"Characterization and Antimicrobial resistance profile of obligate anaerobes associated with orodental infections"

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in

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By

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I, **Dr. Varshith Veeramachaneni** hereby declare that the thesis entitled **"Characterization and Antimicrobial resistance profile of obligate anaerobes associated with orodental infections"** is a bonafide and original work carried out by me under the supervision and guidance of **Dr. Shashwati Nema**, Additional Professor, Department of Microbiology, AIIMS Bhopal. To the best of my knowledge, this thesis or any part thereof has not formed the basis for the award of any degree/fellowship or similar title to any university candidate.

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AIIMS, Bhopal Institutional Human Ethics Committee- Student Research (IHEC-SR) in its 7th meeting held on 22nd February 2023 discussed your proposal titled **"Characterization and antimicrobial resistance profile of obligate anaerobes ssociated with orodental infections"** and evaluated it for suitability for your dissertation as required for fulfilment of MD/MS program.

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1. Prof. D. K. Pal, Former HoD Community Medicine, GMC, Bhopal.....Chairperson
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3. Prof. Amit Agrawal, Professor of Neurosurgery, AIIMS, Bhopal.....Member
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LIST OF ABBREVIATIONS

Notation	Expanded Form
CP	chronic periodontitis
COPD	Chronic obstructive pulmonary disease
HIV/AIDS	Human immunodeficiency virus/ acquired immunodeficiency syndrome
CT	Computed Tomography
MRI	Magnetic Resonance Imaging
MRSA	Methicillin Resistant Staphylococcus aureus
IV	Intra venous
S. anginosus	Streptococcus anginosus
S. constellatus	Streptococcus constellatus
S. intermedius	Streptococcus intermedius
A.israelii	Actinomyces israelii
N. mucosa	Neisseria mucosa
N. subflava	Neisseria subflava
H. influenzae	Haemophilus influenzae
P. gingivalis	Porphyromonas gingivalis
LPS	Lipopolysaccharide
VFA	Volatile fatty acids
SCFA	Short Chain Fatty acids
SOD	Superoxide Dismutase
UV	Ultraviolet
DNA	Deoxyribonucleic acid
m RNA	messenger- Ribonucleic acid
t RNA	transfer-Ribonucleic acid
MLSB	Macrolide Lincosamide Streptogramin B
C. difficile	Clostridioides difficile
m/z ratio	Mass to charge ratio
Kb	Kilobases
FDA	Food and Drug Administration

C. perfringens	Clostridium perfringens
PEA	Phenyl Ethyl Alcohol
ATCC	American Type Culture Collection
MALDI-TOF	Matrix-Assisted Laser Desorption Ionization–Time of Flight
PCR	Polymerase chain reaction
q PCR	quantitative Polymerase chain reaction
NGS	Next-generation sequencing
FISH	fluorescence in situ hybridization
r RNA	ribosomal ribonucleic acid
MIC	Minimum Inhibitory Concentration
CLSI	Clinical and Laboratory Standards Institute
EUCAST	European Committee on Antimicrobial Susceptibility Testing
AMR	Antimicrobial Resistance
E-test	Epsilometer test
AST	Antibiotic susceptibility test
mg/ml	milligrams per millilitre
µg /ml	micrograms per millilitre
CFU	Colony forming unit
µl	Microlitre
SGE	Spiral Gradient endpoint
+nt	Present
-nt	Absent
SES	Socio economic status
DM	Diabetes mellitus
PPV	Positive Predictive Value
NPV	Negative Predictive Value
BBA	Brucella blood agar
KVLB	Kanamycin Vancomycin Laked blood Agar
BA	Blood agar
EYA	Egg Yolk gar
BBE	Bacteroides Bile Esculin (BBE) agar
DMSO	Dimethyl sulfoxide (DMSO)

mg	Milligram
ml	millilitre
S	Susceptible
R	Resistant

INTRODUCTION

INTRODUCTION

Orodental infections are the infections which originate from a tooth or from its supporting structures and can spread to its surrounding tissues.[1] They are the most frequently encountered infections among patients visiting to dental clinics. The common orodental infections are dental caries, periodontitis, dental abscess and pericoronitis.[2,3] Among these dental caries and periodontitis are most commonly encountered infections and have been linked to an increased risk of developing and exacerbating systemic diseases, including cardiovascular disease, bacterial pneumonia and diabetes mellitus, potentially influencing their progression and severity.[4] Dental caries is a bacterial infection that leads to the localized demineralization and degradation of the hard tissues of the teeth, resulting in the progressive destruction of tooth structure. [2] Periodontitis occurs when poor oral hygiene leads to the buildup of plaque and calculus, causing inflammation in the tissues that support the teeth, including the gums, the periodontal ligament, and the surrounding alveolar bone.[1] Failure to properly treat these orodental infections can lead to serious consequences, these infections can spread to the fascial planes of the head and neck, leading to complications such as osteomyelitis. Furthermore, infections originating from the 2nd and 3rd molars can migrate to the submandibular, submental, and sublingual spaces potentially causing Ludwig's angina, a severe and potentially life-threatening condition.[1] In patients with valvular heart disease, bacteria through hematogenous spread leading to bacteremia and potentially causing endocarditis, an infection of the heart valves.[1]

Bacteria colonizing teeth are a major contributor to the pathogens causing these infections..[1] These infections are mostly polymicrobial in nature. Anaerobes make up a significant part of the dental indigenous and pathogenic flora. Anaerobic bacterial infections are a common and potentially life-threatening issue.[1] Despite being the primary cause of orodental infections, obligate anaerobes are often understudied due to various challenges. One major obstacle is the limited availability of specialized culture facilities and technical expertise, making it difficult to isolate and correctly identify these bacteria. Furthermore, identification methods can be expensive and time-consuming. As a result, the role of obligate anaerobes in orodental infections remains less understood.[5]

The treatment of orodental infections relies on a two-fold approach, combining mechanical-surgical management with antibiotic therapy. Mechanical-surgical management involves procedures to remove infected tissue and drain abscesses, while antibiotic therapy targets the underlying bacterial infection. Commonly prescribed antibiotics include metronidazole, ampicillin, clindamycin, tetracycline, and moxifloxacin.[6,7] This comprehensive approach aims to effectively manage and eliminate the infection, promoting optimal oral health outcomes.[8] Dental practitioners often use antimicrobial agents empirically, without waiting for the results of antimicrobial tests, due to the long turnaround time required for culture-dependent tests, especially when dealing with anaerobic bacteria. This approach allows for prompt treatment, as waiting for test results could lead to delayed treatment and potentially worsen the infection. As a result, treatment is often inappropriate and contributes to the growing problem of bacterial resistance, including the emergence of multiple drug-resistance.[9] Over the past few decades, there has been a concerning rise in antimicrobial resistance among anaerobic bacteria, making it increasingly challenging to predict their susceptibility to various antimicrobial agents, and thereby complicating treatment efforts.[10] In view of this the present study is planned to analyze the implicating obligate anaerobes causing dental caries and periodontitis and their current resistance trends for commonly prescribed antimicrobial agents. This will guide the dental practitioners for deciding upon empirical treatment and for selection of appropriate antibiotics.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

ORODENTAL INFECTIONS

Orodental infections are the most common illnesses which are defined as infection of the gums, alveolus, or jaws that starts from a tooth or one of its supporting components. Dental caries and periodontal disease are the most prevalent orodental infections. Other miscellaneous orodental infections include pericoronitis, gingivitis, dental abscess, pulpitis, Ludwig angina. These infections begins locally in the vicinity of a tooth and can either stay in that area or spread to nearby or distant areas.[11]

DENTAL CARIES

It is a bacterial infection that leads to the localized demineralization and degradation of the hard tissues of the teeth, resulting in the progressive destruction of tooth structure.[2] It is a widespread and pervasive oral health issue, affects a significant portion of the population in most industrialized countries, with the majority of both children and adults experiencing the disease at some point in their lives.[12] The development of dental caries is a complex process involving a dynamic interplay between multiple factors, including the tooth's structural composition, the microbial biofilm that forms on its surface, dietary sugars, and individual influences such as saliva composition and genetic predisposition.[13] Globally, untreated dental caries stands as the most common health condition, affecting a staggering 3.1 billion individuals (44% of the world's population).[13] Studies in developing countries such as India report a prevalence rate of 36.7%, while others like Saudi Arabia prevalence to be as high as 83%. [14], resulting in significant impairment to quality of life, substantial financial burdens on individuals, families, and society. Dental caries is linked to a range of local and general risk factors, including wide variety of dietary habits, inadequate oral hygiene practices, irregular brushing routines, and socioeconomic disadvantage, which collectively contribute to the development and progression of the disease.[15] When caries first develops, it only affects the enamel and shows no symptoms. When hot, cold, or sugary foods or drinks come into contact with the affected tooth, as well as when chewing or other pressure is applied, cavities that have

penetrated the dentin create discomfort. When there is significant pulp involvement, pain can be severe.[16,17] Relation to infection progression is influenced by various factors such as biofilm formation, immune response and dietary Factors.[18,19,20] A biofilm composed of acidic environment created by bacterial metabolism promotes the growth of acidogenic and aciduric bacteria, which further exacerbates demineralization.[18] Salivary components, such as antibodies and antimicrobial peptides, help regulate the microbial population in the oral cavity. A compromised immune response can lead to an increased risk of caries.[19] Presence of fermentable carbohydrates in the diet facilitates the growth of cariogenic bacteria, thus promoting infection progression.[20] Despite being largely preventable, dental caries remains a persistent public health issue, with its prevalence showing minimal decline over the past three decades, highlighting a concerning lack of progress in combating this widespread oral health problem.[21]

Majority of the dental caries infections are clinically diagnosed and to ensure comprehensive diagnosis, dental radiographs and other supportive diagnostic methods are necessary as they can reveal caries lesions that are not visible during a visual examination, particularly those situated on the surfaces of adjacent teeth, which can be easily overlooked.[14] The management of dental caries requires a comprehensive approach that encompasses six key objectives: early detection of initial lesions, assessment of caries activity, evaluation of individual risk factors, prevention of new lesions, preservation of dental tissue and prescribing the empirical antimicrobials. By adopting this multifaceted strategy, clinicians can effectively prevent, diagnose, and treat dental caries, ensuring optimal oral health and function for their patients.[22] Among dental caries presence of cavitated lesions, high risk factors like history of frequent caries and patients with co-morbid conditions are the main indications for initiating antimicrobials in dental caries patients.[23] Amoxicillin, amoxicillin-clavulanic acid, metronidazole, secnidazole or clindamycin alone are the commonly prescribed antimicrobials. [24] The prognosis for dental caries depends on various factors, including the patient's overall health, oral hygiene habits, and the extent and severity of the caries.

If detected early, dental caries can be reversed through preventive measures and minor interventions like remineralization, potentially halting the progression of the lesion. However, if left untreated, caries can advance to a moderate stage, resulting in significant tooth loss.[22]

Untreated dental caries can lead to a range of serious complications, including apical periodontitis, an inflammation of the tissues surrounding the tooth root, as well as painful infections such as periapical abscesses and cellulitis.[22] Additionally, dental caries can cause periapical granulomas and cysts to form, and may even lead to osteomyelitis, an infection of the jawbone itself. Furthermore, periostitis, an inflammation of the bone covering, and abscesses, pockets of pus in the surrounding tissues, can also occur. If left unchecked, these complications can have severe consequences for oral health and overall well-being. While dental caries themselves are not typically life-threatening, the infection can spread through facial planes and lead to more severe and potentially life-threatening complications. These include sepsis, a systemic infection, and airway compromise, such as Ludwig angina, a rare but serious condition. Furthermore, odontogenic infections, which originate in the teeth or surrounding tissues, can cause deep neck abscesses, accounting for nearly half (49.1%) of such cases in one study, highlighting the importance of prompt and effective treatment to prevent such serious outcomes.[25]

PERIODONTITIS

It is defined as a chronic bacterial infection that causes inflammation and damage to the periodontal tissues, leading to loss of alveolar bone support and potential tooth loss.[26] Periodontitis is originally a disease originating from the gingival tissue which if left untreated results in penetration of inflammation to the deeper tissues, altering the bone homeostasis causing tooth loss.[27] Periodontal disease is primarily triggered by the colonization of mixed bacteria in the oral tissue, leading to infection and inflammation. However, its progression and severity can be significantly influenced by various secondary factors. These include local factors such as developmental grooves, calculus, dental plaque, overhanging restorations, and anatomical features like short tooth roots and cervical enamel projections. Additionally, systemic factors like genetic predisposition, smoking, stress, and underlying medical conditions can also contribute to the acceleration and exacerbation of the disease. The interplay between these factors determines the severity and progression of the disease.[28,29] Periodontitis can be categorized into two types, chronic periodontitis (CP) and aggressive periodontitis. Chronic periodontitis is characterized by the presence of extensive plaque and calculus buildup, leading to a gradual and persistent inflammation of the periodontal tissues. This type of periodontitis is typically associated with a long-standing history of poor oral hygiene, and its progression is often slow and steady. In contrast, aggressive periodontitis is a

more rapid and severe form of the disease, which can occur even in individuals with good oral hygiene habits.[30] More than half (51%) of the population in India is affected by periodontal disease, making it a widespread and common oral health issue.[26] While chronic periodontitis is most commonly seen in adults, it can also affect younger individuals. Chronic periodontitis is characterized by a gradual and slow progression, primarily driven by the presence of specific microbial bacteria. Unlike aggressive periodontitis, chronic periodontitis is not typically associated with familial aggregation or defects in neutrophil function, suggesting that the underlying causes and risk factors for this condition are distinct.[31] The clinical features of chronic periodontitis, such as attachment loss and gingival bleeding, often go unnoticed by patients due to the disease's painless and asymptomatic nature. This asymptomatic nature of the disease lead to delayed detection and treatment, as patients may not seek dental care until advanced stages of the disease have developed. The lack of noticeable symptoms can make chronic periodontitis a stealthy and insidious condition.[32] Periodontal disease has several consequences that extend beyond local periodontal tissue destruction. The associated increase in C-reactive protein levels has been linked to various systemic conditions, including cardiovascular disease, adverse pregnancy outcomes such as low birth weight in newborns, Type II diabetes mellitus, and chronic obstructive pulmonary disease (COPD).[33] This underscores the significance of periodontal disease as a potential risk factor for a range of systemic health issues, emphasizing the importance of effective periodontitis diagnosis, and management to mitigate these risks and promote overall health and well-being.[34] Relation to infection progression is influenced by various factors such as biofilm formation, immune response and systemic inflammation.[35] Periodontal disease is characterized by the formation of complex microbial biofilms on tooth surfaces. The interaction between bacteria within these biofilms and the host immune response can lead to tissue destruction and disease progression. Periodontitis is linked to systemic inflammatory responses, which can contribute to the progression of other chronic diseases, such as cardiovascular disease and diabetes.[35] Non-surgical management like scaling and root planing are the first steps in the management of periodontitis and is essential for controlling bacterial infection and reducing inflammation.[36] Antimicrobial therapy in periodontitis is indicated in patients with progressive, refractory and recurrent periodontitis. Suggested antibiotics include Tetracycline, Doxycycline, Metronidazole, Clindamycin, Amoxicillin + Clavulanic acid (Augmentin), Azithromycin, Metronidazole + Amoxicillin, and Spiramycin.[36] In aggressive periodontitis, systemic Metronidazole-Amoxicillin combination therapy and other recommended antimicrobials include Tetracycline, Doxycycline, Minocycline, Metronidazole, and Amoxicillin + Clavulanic

acid (Augmentin).[36] The objective of periodontitis management is to enhance the overall health of the gums and preserve the remaining supporting structures of the teeth, thereby halting the progression of periodontal disease and promoting long-term oral health and stability.[34]

OTHER ORODENTAL INFECTIONS

PERICORONITIS

Pericoronitis is characterized by infection and inflammation of the gingival tissue surrounding or covering a partially or newly erupting tooth. This condition predominantly affects the mandibular third molars (wisdom teeth) but can also occur with the eruption of any tooth.[37] This has limited epidemiological data, resulting in varying prevalence rates across studies. However, research suggests that the condition primarily affects young adults, with a reported prevalence of 4.92%. Notably, a strong association exists between pericoronitis and mandibular third molars, with 95% of cases linked to these teeth.[37] The condition's peak incidence coincides with the eruption of third molars, typically occurring between 20-29 years of age. Pericoronitis is primarily associated with several risk factors such as presence of impacted wisdom teeth, particularly partially erupted third molars, is a significant contributor to this condition. Poor oral hygiene plays a crucial role as it facilitates plaque and bacteria accumulation around the affected area, leading to inflammation. Age is also a factor, with young adults in their late teens to early twenties being more susceptible due to the typical eruption timing of third molars Systemic conditions, such as diabetes and immunocompromised states, can further increase susceptibility to pericoronitis. [38 – 41] Symptoms usually includes localizing pain and swelling at the back of the mouth. The Symptoms tend to exacerbate over time. It can also present with an unpleasant taste, halitosis, purulent discharge, limited mouth opening, and difficulty swallowing in more advanced stages.[37] Diagnosis of pericoronitis involves a combination of clinical evaluation and patient history. A thorough examination, including palpation of the affected area, can reveal tenderness and signs of trismus (limited jaw opening). Radiographic imaging is essential to assess the position of the impacted tooth and the surrounding structures help to rule out the disease. Initial management of pericoronitis includes pain relief and symptomatic management by analgesics and infection control by irrigation and cleaning. Antimicrobial therapy is indicated in cases of pericoronitis when there is suspicion of systemic spread of infection. To effectively manage the condition, amoxicillin (500 mg orally every 8 hours for 5 days) or metronidazole (400 mg

orally every 8 hours for 5 days) are recommended as first-line treatments, targeting the causative organisms. However, for patients allergic to penicillin, erythromycin serves as a suitable alternative.[37]

GINGIVITIS

Gingivitis is an inflammatory condition of the gingival tissue most commonly caused by bacterial infection.[38] Gingivitis results from microbial plaque accumulation near the gingival sulcus, with key etiological bacteria including *Streptococcus*, *Fusobacterium, Actinomyces*, *Veillonella*, and *Treponema*. Other potentially implicated species are *Bacteroides, Capnocytophaga, and Eikenella.*[38] Gingivitis exhibits varying prevalence rates across demographics. The condition affects both children and adults, with studies indicating higher prevalence among individuals with lower socioeconomic status, attributed to limited access to healthcare and poor oral health attitudes.[38] Pregnant women are also disproportionately affected, with more severe forms of gingivitis observed in this group. The most common types of gingivitis include plaque-induced, hormonal, acute ulcerative necrotizing, drug-induced, and spontaneously presenting hyperplastic gingivitis, with plaque-induced being the predominant form. Risk factors for this condition includes poor oral hygiene, which is the most significant contributor, as inadequate brushing and flossing allow plaque to accumulate on teeth, leading to inflammation of the gums. Additionally, smoking and tobacco use are well-documented risk factors that impair blood flow to the gums and hinder healing processes. Hormonal changes, such as those occurring during puberty, menstruation, pregnancy, and menopause, can also increase gum sensitivity and susceptibility to inflammation. Certain medical conditions, including diabetes, can exacerbate gum disease due to compromised immune responses and poor wound healing. Medications that cause dry mouth, such as antihistamines and certain antidepressants, can reduce saliva flow, which is vital for maintaining oral health. Clinically, it presents with red, swollen, and tender gums that may bleed easily, especially during brushing or flossing. Gingivitis does not usually cause pain. A hallmark feature is the absence of bone loss or attachment loss, distinguishing it from more advanced periodontal diseases. Bad breath (halitosis) is another common symptom.[38] Diagnosis is based on clinical signs of gum inflammation. Assess the gums for changes in color, texture, and contour, by using a periodontal probe to measure the depth of the gum [42]pockets around the teeth. Pockets greater than 3 mm and tenderness may indicate inflammation. The primary goal of gingivitis management is to reduce inflammation, remove plaque, and establish good oral hygiene

practices to prevent progression. Initial management of gingivitis includes scaling, root planing,[42] Antimicrobials are indicated in the treatment of gingivitis primarily to reduce bacterial load. Antibiotics like metronidazole is used if gingivitis is associated with systemic health issues.[42]

PULPITIS

It is the inflammation of the dental pulp, caused by bacterial infections from tooth decay, trauma, or irritants like repeated dental procedures or tooth cracks, affecting the tooth's innermost tissue comprising of nerves, blood vessels, and connective tissue. Pulpitis affects a substantial population, with 60-90% of school children and virtually all adults experiencing dental cavities, a common precursor to pulpitis. In India, pulpitis predominantly affects molars (60.7%), premolars (68.2%), and anterior teeth (43.8%). [43] Primary risk factors include tooth decay, which allows bacteria to penetrate the pulp and trigger inflammation. Trauma, such as fractures or cracks, and repeated dental procedures that irritate the pulp, also significantly increase the risk of pulpitis. Additionally, poor oral hygiene plays a crucial role, as plaque accumulation accelerates tooth decay and bacterial invasion. Age is another contributing factor, as older individuals often have worn teeth or multiple restorations, making them more susceptible to pulp inflammation.[44,45] Pulpitis presents with a variety of clinical features, primarily characterized by tooth pain, which can range from mild to severe. Pain is usually brief, triggered by stimuli such as cold, sweet, or acidic foods, and subsides quickly after the stimulus is removed. Patients may experience prolonged sensitivity to temperature changes and discomfort when chewing or applying pressure to the affected tooth.[45] Diagnosis is mainly by clinical examination which include percussion and palpation of the gums in assessing the status of gums and supporting structures. Radiographic examinations (X-rays) are essential to visualize the extent of decay and to identify periapical lesions if present. [45] The primary management of pulpitis involves pain management by analgesics and root canal treatment.[45] Antimicrobials are commonly prescribed to prevent complications such as abscess formation The presence of abscesses associated with pulpitis is another critical factor; antibiotics are indicated to control infection, especially when systemic symptoms like fever and swelling are present. Furthermore, immunocompromised patients or those with underlying health conditions, such as diabetes, may require prophylactic or therapeutic antibiotic treatment to prevent severe infection. In instances of severe inflammation, where there is a significant risk of spreading infection, antimicrobials may also be used as adjunctive therapy to address acute

inflammation effectively. Overall, appropriate antimicrobial management is essential for effectively treating pulpitis and preventing complications. The first-line antibiotic commonly used is amoxicillin, known for its broad-spectrum efficacy against the bacteria frequently involved in dental infections. In cases where patients have a penicillin allergy, clindamycin serves as a suitable alternative due to its effectiveness against anaerobic bacteria and its ability to penetrate abscesses. Metronidazole is another valuable option, particularly in managing mixed infections, as it targets anaerobic organisms effectively. Additionally, azithromycin may be prescribed for those with penicillin allergies, providing good coverage against common oral pathogens. [44,45]

COMPLICATIONS OF ORODENTAL INFECTIONS

DENTAL ABSCESS

The primary causes of a dental abscess are dental caries, dental trauma, and inadequate oral hygiene. If left unaddressed, these infections can lead to severe pain and potentially life-threatening complications, including the spread of infection to the deep neck space or intracranial sinuses.[46] When the protective enamel of a tooth is compromised, either through decay, injury, or poor hygiene, it creates an entry point for oropharyngeal bacteria to penetrate the tooth's pulp cavity. This bacterial invasion leads to a localized infection, resulting in the formation of a dental abscess.[47] Several factors can predispose individuals to developing a dental abscess. Partially erupted teeth, such as wisdom teeth, can trap bacteria between the crown and soft tissues, leading to inflammation. Additionally, genetic conditions like amelogenesis imperfecta can weaken tooth enamel, making it more susceptible to wear and decay. Furthermore, exposure to chemical irritants, like smoke from methamphetamine use, and immunosuppression due to chemotherapy or chronic conditions like HIV/AIDS, can increase susceptibility to abscesses. These factors can collectively contribute to the development of dental abscesses by creating an environment conducive to bacterial growth, compromising tooth structure, or impairing the body's ability to fight infection.[48] According to the National Health and Nutrition Examination Survey conducted by the National Centre for Health Statistics, a staggering 91% of adults aged 20 to 64 have experienced dental caries. Approximately, 27% of adults aged 20 to 64 have untreated tooth decay.[47] The initial diagnostic workup for a suspected dental abscess should include imaging studies like computed tomography (CT) and magnetic resonance imaging (MRI) to detect the abscess and evaluate

for possible bacterial spread to the sinus cavities. Treatment of a dental abscess typically involves a multi-faceted approach, including draining the abscess, providing antibiotic support, managing pain, and removing the infectious tooth source. In many cases, oral antibiotics accompanied by a timely intervention can effectively address the issue. Generally, dental abscesses can be successfully treated with antibiotics that target a broad spectrum of bacteria, including gram-negative, facultative anaerobic, and strict anaerobic organisms, thereby resolving the infection and preventing further complications.[46] In the treatment of these infections, penicillins and cephalosporins are commonly used antibiotics. However, the rising concern of antimicrobial resistance due to the production of β-lactamase enzymes has reduced their effectiveness. To combat this resistance, it is now recommended to use penicillins in combination with other antimicrobial agents, such as metronidazole, or to opt for antibiotics with an extended spectrum of activity, like ampicillin-sulbactam or ampicillin-clavulanate. However, if left untreated, the prognosis can deteriorate significantly, leading to potentially life-threatening complications. In severe cases, where the infection descends and causes mediastinitis, the mortality rate can surge to an alarming 40%. If the infection enters the bloodstream, it can cause sepsis, a systemic infection that can lead to organ failure and death.[47,48]

LUDWIG'S ANGINA

It is a rare and potentially life-threatening condition characterized by a diffuse cellulitis infection that affects the soft tissues of the floor of the mouth and neck.[49] Ludwig's angina primarily arises from dental infections in the lower molars, specifically the second and third mandibular molars, which are responsible for approximately 90% of reported cases. The primary source of infection is typically periapical abscesses forming around the second or third mandibular molars.[49] The condition most commonly affects individuals between the ages of 20 and 50, although it can occur at any age. Risk factors include dental infections, particularly involving molars, are a significant risk factor for Ludwig's angina Poor oral hygiene, recent dental procedures, weakened immune systems (e.g., diabetes, immunosuppression), and lifestyle factors (tobacco, alcohol use) further increase susceptibility.[50] Ludwig's angina presents with bilateral submandibular and sublingual swelling, elevating the mouth floor, accompanied by severe pain, dysphagia, and dysphonia. If left untreated, severe cases can lead to airway obstruction and respiratory distress.[50] Diagnosis involves clinical evaluation

(patient history, physical exam), imaging studies (CT scans), and laboratory tests (white blood cell counts).[50] Treatment of Ludwig's angina focuses on securing the airway to prevent asphyxiation, followed by intravenous broad-spectrum antibiotics to control infection, and surgical drainage of abscesses to resolve the infection.[49] After securing the airway, Ludwig's angina treatment involves broad-spectrum IV antibiotics covering aerobic, anaerobic, and oral microflora. Ampicillin-sulbactam or clindamycin are common choices. Immunocompromised patients require extended coverage with cefepime, meropenem, or piperacillin-tazobactam, and potentially MRSA coverage with vancomycin IV or linezolid IV. [49]

MICROBIOME OF ORAL CAVITY

The human oral cavity harbours over 300 diverse bacterial species, with approximately 10 gram-negative anaerobic species identified as key periodontal pathogens in recent years. This includes various species of streptococci, such as those belonging to the viridans group (encompassing the anginosus group, formerly known as "*Streptococcus milleri*" group, which includes *S. anginosus*, *S. constellatus*, and *S. intermedius*, along with a range of non-spore-forming anaerobic bacteria, all of which contribute to the complex ecosystem of the oral cavity. Anaerobic bacteria contribute to the breakdown of organic compounds in the mouth, such as carbohydrates and proteins. This process helps maintain a dynamic balance of nutrients in the oral cavity, supporting the overall health of the microbiome.[51] The process of bacterial colonization commences shortly after birth, unfolding in a sequential manner, with a steady expansion of species diversity.[51] This complex microbial ecosystem encompasses a broad spectrum of non-spore-forming anaerobes, including gram-negative species such as *Prevotella, Bacteroides, Porphyromonas, Fusobacterium, Campylobacter, and Veillonella* and gram positive species such as *Actinomyces, Bifidobacterium, Collinsella, Eggerthella, Eubacterium, Lactobacillus, Pseudoramibacter, Slackia, Peptostreptococcus, Micromonas,* and *Finegoldia.* As this diverse microbial community evolves, it contributes significantly to the development and maturation of the human microbiome.[51]

BACTERIAL AETIOLOGY FOR ORODENTAL INFECTIONS

Orodental infections are primarily periodontal diseases and dental caries, result from chronic bacterial infections originating from the normal oral flora. When the bacterial load surpasses the host's defensive capabilities, disease occurs. Orodental infections encompasses a diverse

range of aerobic and anaerobic bacteria, including Streptococcus species (*S. sanguinis and S. mitis*), which play a crucial role in dental plaque formation, leading to caries and periodontitis. Significant aerobic bacteria include *Staphylococcus aureus, Actinomyces species* (*A. israelii*), *Neisseria species* (*N. mucosa* and *N. subflava*), *Corynebacterium species, Haemophilus species* (*H. influenzae*), and *Pseudomonas aeruginosa*.[51- 53] These bacteria can contribute to various orodental infections, particularly in immunocompromised individuals or when the oral environment is disrupted. *Staphylococcus aureus*, which is commonly associated with abscess formation and soft tissue infections, particularly in immunocompromised individuals, and *Enterococcus faecalis*, frequently linked to persistent root canal infections.[51] *Pseudomonas aeruginosa*, though typically an opportunistic pathogen, can colonize the oral cavity, especially in hospital settings, causing infections in vulnerable patients. Additionally, *Neisseria* species, usually part of the normal oral flora, can contribute to infections when oral hygiene is compromised.[51,52] While anaerobic bacteria dominate in chronic conditions, these aerobic species play a crucial role in the acute phase and can significantly influence the progression of orodental infections. Notably, the interplay between aerobic and anaerobic microorganisms often results in polymicrobial infections, complicating treatment and management strategies. Understanding this complex microbial profile is essential for developing targeted therapies and improving patient outcomes in orodental infections.[54] Conversely, gram-positive, facultatively anaerobic mutans streptococci are the primary etiological agents responsible for dental caries.[55] Orodental infections are characterized by an abundance of anaerobic bacteria, particularly spirochetes and black-pigmented species like *Porphyromonas gingivalis* and *Prevotella intermedia*. While *P. gingivalis* is often implicated in early-onset periodontitis, *P. intermedia* is found in both early-onset and acute forms of the disease. [56] Approximately 20% of orodental infections are caused exclusively by strict anaerobes. The polymicrobial nature of these infections, combined with the presence of cultivable and uncultivable microbes, poses a significant challenge for diagnostic analysis in routine microbiological laboratories.[57] The primary anaerobic bacteria isolated from orodental infections predominantly from the dental caries and periodontitis belong to the genera *Prevotella, Fusobacterium* and *Porphyromonas*. Notably, *Prevotella species* are the most frequently detected, present in 10-87% of cases. Specific pathogens commonly identified include *Prevotella intermedia, Prevotella nigrescens, Prevotella pallens, Porphyromonas endodontalis, and Porphyromonas gingivalis*.[57] *Fusobacterium species*, particularly *Fusobacterium periodonticum* and *Fusobacterium nucleatum*, are commonly associated with periodontitis.[57]

ROLE OF OBLIGATE ANAEROBES IN ORODENTAL INFECTIONS

Anaerobic infections typically occur at the same sites where anaerobic microorganisms normally colonize and reside, highlighting the transition from a commensal to a pathogenic relationship. Anaerobes are microorganisms that thrive in environments devoid of oxygen and are unable to survive in its presence.[54] The toxicity of oxygen to anaerobic microorganisms can be attributed to their lack of essential enzymes, including catalase, superoxide dismutase, and peroxidase. These enzymes play a crucial role in protecting aerobic organisms from the harmful effects of oxygen by neutralizing reactive oxygen species. Without these enzymes, anaerobes are unable to defend themselves against the damaging effects of oxygen, making it a toxic environment for them to survive.[58]

Anaerobic microorganisms play a crucial role in the orodental infections, comprising a substantial portion of both the indigenous and pathogenic flora. They are involved in various orodental infections, including periodontal disease, dental caries and infections of the hard and soft oral tissues. Moreover, anaerobes can serve as a focal point for disseminated infectious diseases, underscoring their significance in both oral and systemic health. Their role in these infections is well established, and understanding their involvement is essential for effective diagnosis and treatment.[55] Anaerobes which are associated with oral infections are *Actinobacillus actinomycetem-comitans, Porphyromonas gingivalis,* and *Prevotella intermedia* are among the bacteria that can contribute to these infections.[59] Additionally, *Bacteroides forsythus, Campylobacter rectus, Eubacterium species, Fusobacterium nucleatum, Eikenella corrodens, and Peptostreptococcus micros* have also been implicated in oral infections.[59] The leading causes of tooth loss in both children and adults are dental caries and periodontal infections, with *Streptococcus mutans* being the main bacterial offender responsible for dental caries. This bacterium is a key contributor to the development of tooth decay. In healthy individuals, the normal oral flora was found to be dominated by anaerobic gram-positive cocci, with *Streptococcus sanguis* being the predominant species. In contrast, dental caries were characterized by anaerobic gram-positive bacilli, with *Streptococcus mutans* emerging as the primary species. Furthermore, periodontitis, a severe gum infection, was associated with a diverse array of microorganisms, including gram-negative bacilli, black-pigmented *Bacteroides, Fusobacterium*, and *Aggregatibacter actinomycetemcomitans*.[2] Obligate gram-negative anaerobes were predominantly isolated in patients with Chronic

Periodontitis, outnumbering Gram-positive anaerobes.[60] The most common anaerobic isolates in Chronic Periodontitis patients were *Prevotella spp*.(60%), *Fusobacterium spp*. (50*%), Porphyromonas spp*. (30%), and *Bacteroides spp*. (25%), with significantly lower isolation rates in healthy subjects (30%, 15%, 10%, and 10%, respectively).[60] These findings underscore the critical role of anaerobic microorganisms, particularly Gram-negative species, in the pathogenesis of Chronic Periodontitis.[60]

VIRULANCE FACTORS

These pathogens contribute to orodental infections through various virulence factors. They produce polysaccharide capsules, fimbriae, and adhesins to evade phagocytosis, adhere to host tissues, and form protective biofilms. They also possess proteolytic enzymes, such as gingipains in *P.gingivalis*, which degrade host proteins like collagen and immune components, promoting tissue destruction and nutrient acquisition. Additional virulence factors include hemolysins, cytotoxins, lipopolysaccharides (LPS), volatile fatty acids (VFAs), and short-chain fatty acids (SCFAs), which trigger inflammation, damage host cells, and impair immune function. Moreover, superoxide dismutase (SOD) enables anaerobes to withstand oxidative stress, while hydrolytic enzymes like hyaluronidase and collagenase degrade host tissues, facilitating infection spread. [61-66]

CLASSIFICATION OF OBLIGATE ANAEROBES

A. TAXONOMIC CLASSIFICATION

Table 1: Taxonomic classification of clinically important obligate anaerobic gram-positive cocci.[67-74]

Phylum and genus	Species or subspecies	Previous name(s) or synonym
Firmicutes		
*Anaerococcus**	*A. hydrogenalis*	*Peptostreptococcus hydrogenalis*
	A. lactolyticus	*Peptostreptococcus lactolyticus*
	A. octavius	*Peptostreptococcus octavius*
	A. prevotii	*Peptostreptococcus prevotii*

Phylum and genus	Species or subspecies	Previous name(s) or synonym
	A. tetradius	*Peptostreptococcus tetradius*
	A. vaginalis	*Peptostreptococcus vaginalis*
*Anaerosphaera**	*A. aminiphila*	*New species*
*Atopobium**	*A. parvulum*	*Streptococcus parvulus*
*Blautia**	*B. producta*	*Peptostreptococcus productus, Ruminococcus productus*
*Finegoldia**	*F. magna*	*Peptostreptococcus magnus*
*Gallicola**	*G. barnesae*	*Peptostreptococcus barnesae*
*Murdochiella**	*M. asaccharolytica*	*New species*
*Parvimonas**	*P. micra*	*Peptostreptococcus micros, Micromonas micros*
Peptococcus	*P. niger*	
*Peptoniphilus**	*P. asaccharolyticus*	*Peptostreptococcus asaccharolyticus*
	P. harei	*Peptostreptococcus harei*
	P. indolicus	*Peptostreptococcus indolicus*
	P. ivorii	*Peptostreptococcus ivorii*
	P. lacrimalis	*Peptostreptococcus lacrimalis*
Peptostreptococcus	*P. anaerobius*	

Table 2: Taxonomic classification of clinically important obligate anaerobic gram-positive bacilli [75-91]

Phylum and genus	Species or subspecies	Previous name(s) or synonym
Actinobacteria		
*Actinobaculum**	*A. massiliense*	*Actinomyces massiliae*
	A. suis	*Actinomyces suis*

Phylum and genus	Species or subspecies	Previous name(s) or synonym
Actinomyces	*A. johnsonii*	*New species, A. naeslundiigenospecies WVA 963*
	A. naeslundii	*A. naeslundii genospecies 1*
	A. oris	*New species, A. naeslundiigenospecies 2*
*Actinotignum**	*A. sangunis*	*New species*
	A. schaalii	*Actinobaculum schaalii*
	A. urinale	*Actinobaculum urinale*
*Alloscardovia**	*A. omnicolens*	*New species*
Bifidobacterium	*B. logumsubspecies infantis*	*New subspecies, B. infantis*
	B. logumsubspecies longum	*New subspecies, B. logum*
	B. logumsubspecies suis	*New subspecies, B. suis*
*Collinsella**	*C. aerofaciens*	*Eubacterium aerofaciens*
*Cutibacterium**	*C. acnes*	*Propionibacterium acnes*
	C. avidum	*Propionibacterium avidum*
	C. granulosum	*Propionibacterium granulosum*
	C. humersii	*Propionibacterium humersii*
*Paraeggerthella**	*P. hongkongenesis*	*Eggerthella hongkongenesis*
*Parascardovia**	*P. denticola*	*Bifidobacterium denticolens*
*Pseudopropionibacterium**	*P. propionicum*	*Propionibacterium propionicum*
*Scardovia**	*S. inopinata*	*Bifidobacterium inopinatum*
Firmicutes		
*Anaerostipes**	*A. hadrus*	*Eubacterium hadrum*
Atopobium	*A. minutus*	*Lactobacillus minutus*
	A. rimae	*Lactobacillus rimae*
*Clostridioides**	*C. difficile*	*Clostridium difficile*
Clostridium	*C. maximum*	*Sarcina maxima*
	C. moniliforme	*Eubacterium moniliforme*

Phylum and genus	Species or subspecies	Previous name(s) or synonym
	C. tarantellae	*Eubacterium tarantellae*
	C. ventriculi	*Sarcina ventriculi*
Dorea	*D. formicigenerans*	*Eubacterium formicigenerans*
Eubacterium	*E. sulci*	*Fusobacterium sulci*
Filifactor	*F. alocis*	*Fusobacterium alocis*
*Hathewaya**	*H. histolytica*	*Clostridium histolyticum*
	H. limosa	*Clostridium limosum*
	H. proteolytica	*Clostridium proteolyticum*
*Hungatella**	*H. hathewayi*	*Clostridium hathewayi*
*Paeniclostridium**	*P. sordellii*	*Clostridium sordellii*
*Paraclostridium**	*P. bifementans*	*Clostridium bifementans*
*Terrisporobacte**	*T. glycolicus*	*Clostridium glycolicum*

Table 3: Taxonomic classification of clinically important obligate anaerobic gram-negative bacilli [92-100]

Phylum and genus	Species or subspecies	Previous name(s) or synonym
Bacteroidetes		
Bacteroides	*B. fragilis group*	
Parabacteroides	*P. chongii*	*New species*
	P. distasonis	*Bacteroides distasonis*
	P. godonii	*New species*
	P. goldsteinii	*Bacteroides goldsteinii*
	P. johnsonii	*New species*
	P. merdae	*Bacteroides merdae*
Porphyromonas	*P. asaccharolytica*	*Bacteroides asaccharolyticus*
		Bacteroides melaninogenicasubspecies asacc h-arolytica
	P. endodontalis	*Bacteroides endodontalis*
	P. gingivalis	*Bacteroides gingivalis*
	P. pasteri	*New species*
	P. pogonae	*New species*
Prevotella	*P. bivia*	*Bacteroides bivius*
	P. buccae	*Bacteroides buccae, Bacteroides ruminicola subspecies brevis*

Phylum and genus	Species or subspecies	Previous name(s) or synonym
		Bacteroides capillus, Bacteroides pentosaceus
	P. buccalis	*Bacteroides buccalis*
	P. corporis	*Bacteroides corporis*
	P. dentalis	*Mitsuokella dentalis, Hallella seregens*
	P. denticola	*Bacteroides denticola*
	P. disiens	*Bacteroides disiens*
	P. heparinolytica	*Bacteroides heparinolyticus, related to B. fragilis group*
	P. intermedia	*Bacteroides intermedius*
		Bacteroides melaninogenicasubspecies inter medius
	P. micans	*New species*
	P. loescheii	*Bacteroides loescheii*
	P. melaninogenica	*Bacteroides melaninogenica*
		Bacteroides melaninogenicasubspecies mela ninogenicus
	P. nanceiensis	*New species*
	P. oralis	*Bacteroides oralis*
	P. oris	*Bacteroides oris, Bacteroides ruminicola subspecies brevis*
	P. oulorum	*Bacteroides oulorum, Prevotella oulora*
	P. veroalis	*Bacteroides veroalis*
	P. zoogleoformans	*Bacteroides zoogleoformans, related to B. fragilis group*
Fusobacteria		
Fusobacterium	*F. equinum*	*New species*
	F. nucleatumsubspecies an imalis	
	F. nucleatumsubspecies ne cleatum	
	F. nucleatumsubspecies po lymorphum	

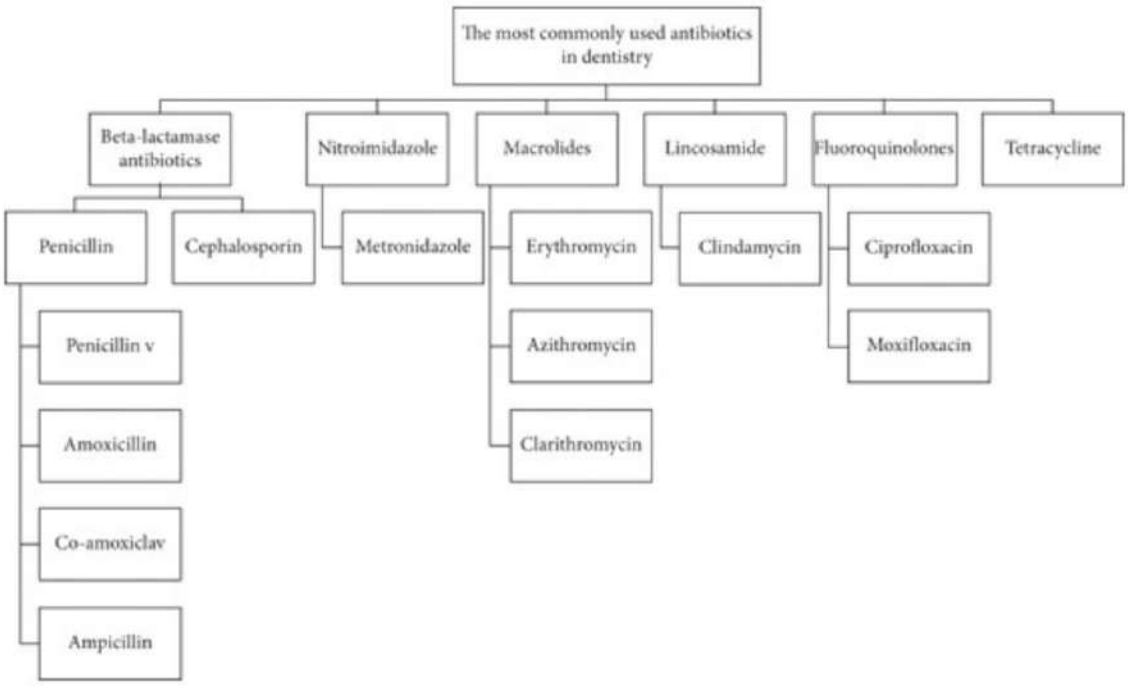
Phylum and genus	Species or subspecies	Previous name(s) or synonym
	F. nucleatumsubspecies vincentii	*Later synonym of F. nucleatumsubspecies fusiform*
	F. pseudoperiodonticum	*New species*
Dialister	*D. invisus*	*New species*
	D. pneumosintes	*Bacteroides pneumosintes*
*Selenomonas**	*S. sputigena*	
Proteobacteria		
*Bilophila**	*B. wadsworthia*	*New species*
*Sutterella**	*S. wadsworthensis*	*Campylobacter gracilis, Bacteroides gracilis*

B. Table 4: CLASSIFICATION BASED ON GRAM REACTION AND MORPHOLOGY [101]**:**

Classification	Obligate anaerobes
Gram positive anaerobic cocci	*Micromonas*
	Peptococcus
	Peptostreptococcus
	Parvimonas
	Peptoniphilus
	Coprococcus
	Ruminococcus
	Abiotrophia
	Atopobium
	Gemella
	Granulicatella
	Streptococcus
	Finegoldia
Gram positive anaerobic bacilli	
Spore forming	*Clostridium*
	Filifactor
Non-spore forming	
	Propionibacterium
	Corynebacterium matruchotii

	Bifidobacterium
	Lactobacillus
	Actinomyces
	Bulleidia
	Collinsella
	Rothia
	Mogibacterium
	Eggerthella
	Eubacterium
	Slackia
Gram negative anaerobic cocci	
	Veillonella
	Acidaminococcus
	Megasphaera
Gram negative anaerobic bacilli	
	Fusobacterium
	Leptotrichia
	Desulfomonas
	Bacterioides
	Bilophila
	Campylobacter(gracilis)
	Catonella
	Dialister
	Dichelobacter
	Fibrobacter
	Megamonas
	Porphyromonas
	Prevotella
	Rikenella
	Ruminobacter
	Sebaldella
	Sutterella
	Tissierella
	Butyrivibrio
	Succinivibrio
	Anaerovibrio
	Desulfovibrio
	Selenomonas
	Anaerobiospirillum

COMMONLY PRESCRIBED ANTIMICROBIALS IN ORODENTAL INFECTIONS [102,103]



1. β-lactam Antibiotics [104]

In order to manage and treat a variety of illnesses caused by anaerobic bacteria, β-lactam antibiotics play's an essential role. These are among the most frequently prescribed medications, boasting a wide range of clinical applications and indications.

β-lactam antibiotics comprise several classes, each with distinct structural features and properties:[104]

1. Penicillins

- Contain a 6-aminopenicillanic acid ring nucleus with various side chains
- Subclasses
  - Natural penicillins
  - Beta-lactamase-resistant agents
  - Aminopenicillins (Ampicillin)
  - Carboxypenicillins
  - Ureidopenicillins

2. Cephalosporins

• Feature a 7-aminocephalosporanic acid nucleus with a 3,6-dihydro-2H-1,3-thiazane ring side chain

• Traditionally divided into five generations

Spectrum of β-lactam Antibiotics in Anaerobic Orodental Infections:

Penicillins

Spectrum: [105,106]

Penicillins, including penicillin G and penicillin V, have traditionally been effective against gram-positive anaerobic bacteria, such as *Peptostreptococcus*, and certain gram-negative anaerobes. However, their effectiveness is diminished in the presence of beta-lactamase-producing bacteria, including *Bacteroides fragilis*, *Prevotella* species which can inactivate penicillins.

Cephalosporins

Spectrum: [107]

- Second-generation cephalosporins, such as cefuroxime, exhibit moderate activity against anaerobic bacteria, including *Prevotella* and *Porphyromonas species*. However, their effectiveness may be limited against beta-lactamase-producing organisms unless combined with agents like metronidazole.
- Third-generation cephalosporins, like ceftriaxone, offer broader coverage for anaerobic orodental infections

Mechanism of action [104]

β-lactam antibiotics target peptidoglycan, a vital bacterial cell wall component. They inhibit peptidoglycan synthesis by acylating transpeptidases and binding to penicillin-binding proteins, disrupting cross-linking and inducing bacterial lysis or death.

Mechanisms of Resistance [108]

β-lactam agents are the most effective anti-anaerobic antimicrobials. When bacteria develop resistance to penicillins, combining a penicillin with a β-lactamase inhibitor can often overcome this resistance. Three primary mechanisms are responsible for the resistance to β-lactam antibiotics.

a. Change in the target site, leading to reduced binding affinity for the β-lactam agent
b. Decreased permeability
c. Presence of an inactivating enzyme

2. Nitroimidazole

Metronidazole

Spectrum: [109]

Exhibit in vitro potency against anaerobic bacteria. Metronidazole, in particular, demonstrates exceptional in vitro activity against a wide range of obligate anaerobic bacteria, including *Bacteroides fragilis group, Bacteroides species, Fusobacteria, Clostridia etc.* If a polymicrobial infection is being treated, an additional antimicrobial agent (such as a cephalosporin or fluoroquinolone) that targets aerobic bacteria is required, as metronidazole lacks efficacy against these organisms.

Mechanism of action [110]

Metronidazole's mechanism of action involves a four-step process:

1. Diffusion: Metronidazole enters the organism by diffusing across the cell membranes of both anaerobic and aerobic pathogens, but its antimicrobial effects are specific to anaerobes.
2. Reductive activation: Intracellular transport proteins alter the chemical structure of pyruvate-ferredoxin oxidoreductase, reducing metronidazole and creating a concentration gradient that drives the uptake of more drug and promotes the formation of cytotoxic free radicals.
3. Interaction with intracellular targets: Cytotoxic particles interact with host cell DNA, causing DNA strand breakage and fatal destabilization of the DNA helix.
4. Breakdown of cytotoxic products: The final step involves the degradation of cytotoxic products, ultimately leading to the death of the anaerobic pathogen.

Mechanism of Resistance [111-113]

Metronidazole resistance is frequently linked to decreased nitroreductase activity and medication absorption. A decrease in the reducing environment within the cell will lead to decreased nitroreductase activity as well as decreased medication absorption since entry of 5-nitroimidazoles into the cell depends on the rate of reduction of the nitro group.(111) There are two known genes, nimA and nimB, that can give moderate-to-high levels of metronidazole resistance.(112) After screening a set of *B. fragilis* isolates resistant to metronidazole, it was discovered that each isolate carried DNA sequences that hybridized with either a nimA or nimB DNA probe. In contrast, none of the isolates that were susceptible to metronidazole had DNA

sequences that crossed-hybridized with either a nimA or nimB DNA probe.(113) The nimA and nimB genes have been found on both the chromosome and in multiple plasmids. The plasmids containing the nimA or nimB gene have not been shown to be self-transmissible, however these plasmids can be mobilized by other conjugal elements or acquired via transformation.

3. Macrolides

Spectrum: [109]

Macrolide antibiotics, known for their favorable safety profile with minimal toxicity in humans exhibit moderate to good in vitro efficacy against a range of anaerobic bacteria, with the exception of strains within the *Bacteroides fragilis* group and *Fusobacterium species*, which tend to be less susceptible. Macrolides exhibit a spectrum of activity against various anaerobic bacteria, with notable effectiveness against pigmented *Prevotella* and *Porphyromonas species*, microaerophilic *streptococci*, and certain gram-positive anaerobic bacilli and *Clostridium species*. Nevertheless, their activity is diminished against *Fusobacterium* and *Peptostreptococcus species*, highlighting the importance of targeted antibiotic selection. Clarithromycin stands out as the most effective macrolide against a range of gram-positive oral anaerobes, demonstrating superior activity against *Actinomyces, Propionibacterium, Lactobacillus, and Bifidobacterium dentium.*

Mechanism of action [114]

Macrolides exert their antibacterial effect by inhibiting protein synthesis in bacteria. Their mechanism of action involves binding to the 50S subunit of the bacterial ribosome, thereby halting the production of essential proteins. By binding to the ribosome, macrolides prevent the translation of messenger RNA (mRNA) into proteins, specifically by blocking the action of peptidyltransferase, an enzyme responsible for adding amino acids to the growing peptide chain. This inhibition occurs by preventing the attachment of the next amino acid, carried by transfer RNA (tRNA), to the peptide chain, effectively terminating protein synthesis

Mechanism of Resistance [115]

Macrolide resistance arises from various mechanisms that enable bacteria to evade the antibacterial effects of these agents. One primary mechanism involves target site modification, where methylation of the 23S rRNA by erm (erythromycin ribosome methylation) enzymes reduces macrolide binding.[115] Additionally, mutations in the 23S

rRNA sequence can alter macrolide binding, rendering the antibiotic ineffective. Another key mechanism involves active efflux, where macrolide-specific efflux pumps, such as MexAB-OprM and Msr(A), expel macrolides from the bacterial cell. Enzymatic degradation also plays a role, with macrolide-inactivating enzymes like esterases and kinases degrading or modifying macrolides. Plasmid-mediated resistance is another significant mechanism, where transferable resistance genes like mef(A) and erm(B) confer macrolide resistance.[115] Furthermore, reduced permeability due to changes in outer membrane proteins can decrease macrolide uptake, while biofilm formation shields bacteria from macrolide effects. Specific resistance mechanisms include MLSB (Macrolide-Lincosamide-Streptogramin B) resistance, where target site modification and efflux pumps confer resistance to multiple macrolides.[115] The M phenotype, characterized by macrolide-resistant bacteria lacking erm genes, is another notable mechanism. Several factors contribute to the development of macrolide resistance, including overuse and misuse of macrolides, horizontal gene transfer, and high mutation rates.

4. Lincosamide

Spectrum: Clindamycin boasts a comprehensive spectrum of activity against anaerobic microorganisms, demonstrating its effectiveness in numerous clinical trials. As a result, it has become a valuable treatment option for dental infections, particularly in individuals with a penicillin allergy, providing a reliable alternative for managing these infections. The concern with clindamycin is *C. difficile*-associated colitis.

Mechanism of action [116]

Clindamycin exerts its antibacterial effect by inhibiting protein synthesis, specifically by preventing the formation of peptide bonds. This is achieved through its reversible binding to the 50S subunit of bacterial ribosomes, thereby blocking the synthesis of essential proteins.

Mechanism of Resistance [117]

Clindamycin resistance has been linked to the three traditional routes of antibiotic resistance such as drug inactivation, changed permeability, and altered target site. The latter mechanism, known as streptogramin B (MLS)/macrolide/lincosamide resistance, is the most prevalent mechanism of clindamycin resistance in Bacteroides species. Methylation of the 23S rRNA at one of two adenine residues in staphylococci mediates resistance. Because of this methylation, clindamycin is unable to bind to some ribosomes effectively, making them

resistant to the drug's inhibitory effects.(117) It was discovered that conjugal transfer of clindamycin resistance is mediated by plasmids when it was initially reported in 1979 by scientists at three different labs. Many of these plasmids have a size range of 14.6 kilobases (kb) and are self-transmissible.(117) It has also been shown that clindamycin resistance is chromosomally encoded. Chromosome resistance to clindamycin has been connected to tetracycline resistance in a number of instances.(117)

5. Fluoroquinolones

Spectrum: [109]

Quinolones can be categorized based on their antianaerobic activity:

Low activity: ciprofloxacin, ofloxacin, levofloxacin, fleroxacin, pefloxacin, enoxacin, and lomefloxacin

Intermediate activity: sparfloxacin and grepafloxacin

The FDA has approved moxifloxacin as a standalone treatment for skin and soft tissue infections in adults, based on clinical studies demonstrating its effectiveness against a range of anaerobic pathogens. The increasing resistance of *Bacteroides fragilis* group species to fluoroquinolones has led to reservations among clinicians about using moxifloxacin as a treatment option for anaerobic infections, despite its FDA approval. The use of quinolones in paediatric patients is limited due to potential detrimental effects on cartilage development. Meanwhile, the expansion of fluoroquinolone use to treat anaerobic infections is hindered by two primary concerns:

1. Rising resistance among *Bacteroides fragilis* group strains and anaerobic gram-positive cocci.
2. The potential contribution of fluoroquinolones to the growing incidence of Clostridioides difficile-associated disease.

Mechanism of action [118]

Quinolone antibiotics exert their effect by inhibiting DNA synthesis through the targeting of two crucial type II topoisomerases: DNA gyrase and topoisomerase IV (Topo IV). These enzymes facilitate the passage of one double-stranded DNA molecule through another, followed by the rejoining of the original strand, resulting in a two-unit change in the DNA linking number per enzymatic step. Despite their structural and functional similarities, DNA gyrase and Topo IV have distinct roles during DNA replication.

Mechanism of Resistance [111]

Fluoroquinolone resistance arises from various mechanisms that enable bacteria to evade the antibacterial effects of these agents. The primary mechanisms include target modification, efflux pumps, and plasmid-mediated resistance. Target modification involves mutations in the target enzymes, DNA gyrase and topoisomerase IV, which alter the binding site for fluoroquinolones, reducing their affinity. Efflux pumps, such as NorA and MexAB-OprM, actively expel fluoroquinolones from the bacterial cell, while plasmid-mediated resistance involves the transfer of resistance genes, like qnr, that confer fluoroquinolone resistance.

6. Tetracyclines [109]

Spectrum

Tetracycline, previously the preferred treatment for anaerobic infections, has become less effective due to widespread resistance among anaerobic bacteria, including *Bacteroides* and *Prevotella species*. As a result, its usefulness is now limited. The newer tetracycline analogues, doxycycline and minocycline, exhibit enhanced activity compared to the original compound. However, due to widespread resistance, their use is now limited to cases where susceptibility testing can be conducted or in mild to moderate infections where a therapeutic trial is possible.

Mechanism of action [119]

Tetracyclines exert their antibacterial effect by specifically targeting the 30S ribosomal subunit, preventing the attachment of aminoacyl-tRNA to the mRNA-ribosome complex at the acceptor site. This inhibition disrupts protein synthesis, crippling the cell's ability to function, grow, and replicate.

Mechanism of Resistance

The only documented mechanism of tetracycline resistance is modification or protection of the target site. The tetQ gene codes for a protein that can protect the ribosomal protein synthesis machinery against the antibiotics in the tetracycline class that impede protein synthesis.[111] The majority of tetracycline-resistant isolates had a tetQ or tetQ-related gene, according to DNA cross-hybridization studies. Tetracycline resistance, however, may also be caused by another mechanism (such as tetracycline efflux) or a different family of ribosomal protection proteins, as evidenced by the discovery of tetracycline-resistant isolates lacking tetQ DNA sequences. It has recently been determined that *C. perfringens* possesses two tetracycline resistance genes. The genes tetA(P) and tetB(P) combine to produce an operon that codes for two independent proteins that confer resistance to tetracyline through two distinct pathways. A

tetracycline efflux pump is produced by the tetA(P) gene, whereas a protein that confers tetracycline resistance at the ribosome level is encoded by the tetB(P) gene.[120] The tetQ gene-mediated tetracycline resistance is both transferable and inducible.

Table 5 : Type of Orodental infection and treatment agents [121]

Infection	Pathogenic microorganism	First-line therapy	Adjunct therapy
Dental caries	*Streptococcus mutans, Actinomyces species, lactobacilli.*	Metronidazole + Amoxicillin or Co-Amoxiclav	Clindamycin
Periodontitis	*Porphyromonas gingivalis, bacteroid forsythias, Actinobacillus, prevotella intermedia, fusobacterium nucleatum*	Tetracycline	Doxycycline or minocycline
Pericoronitis	*Peptostreptococcus micros, Porphyromonas gingivalis, fusobacter species*	Metronidazole	Amoxicillin
Gingivitis	*Campylobacter rectus, Actinomyces, prevotella intermedia, streptococcus anginosus*	Tetracyclines (tetracycline doxycycline or minocycline)	Amoxicillin or metronidazole
Pulpitis	*Fusobacterium nucleatum, prevotella intermedia, Peptostreptococcus micros, capnociytophaga achalasia, Serenoa endodontics*	Amoxicillin or Azithromycin	Cefoxitin
Dental abscess	*Bacteroides, fusobacterium, Actinomyces, Peptostreptococcus, Peptococcus, Porphyromonas, prevotella oralis, and melanogenic*	Ampicillin-sulbactam, amoxiclav. Penicillin G plus metronidazole or Cefoxitin	Clindamycin plus metronidazole

Infection	Pathogenic microorganism	First-line therapy	Adjunct therapy
Ludwig angina	This disease is a polymicrobial infection caused by oral bacteria, including Streptococcus, Staphylococcus, Peptostreptococcus, Fusobacterium, Bacteroides, and Actinomyces.	Ampicillin-sulbactam or clindamycin	Cefepime, Meropenem or piperacillin-tazobactam to cover pseudomonas aeroginosa in immunosuppressed patients.

LABORATORY DIAGNOSIS OF OBLIGATE ANAEROBES IN CASE OF ORODENTAL INFECTIONS [123-128]

SAMPLE COLLECTION [123,124]

Orodental Infections	Acceptable Samples
Dental caries	Carious Material
Periodontitis	Plaques
Pericoronitis	Crevicular Fluid
Gingivitis	Gingival Crevicular Fluid / Gingival Tissue
Pulpitis	Pulp Tissue / Pus Aspirate
Dental abscess	Pus Aspirate

TRANSPORTATION [125- 128]

- **Use of Anaerobic Transport Media**: After sample collection, specimens must be placed immediately into anaerobic transport media to maintain the low-oxygen conditions required for the survival of obligate anaerobes. Transport media such as robertson's cooked meat media, thioglycollate broth or commercial anaerobic transport systems (e.g., BD Port-A-Cul, Oxoid Anaerobic Transport Medium) help maintain anaerobic conditions during transit. Transport should be quick and at room temperature, as delays may lead to a reduced number of viable organisms.

- **Proper Sealing and Storage**: Samples should be securely sealed in the transport containers to prevent oxygen exposure. These containers are often filled with an oxygen-free gas or contain reducing agents that scavenge oxygen to maintain the anaerobic environment.

DIRECT MICROSCOPY [129-133]

Direct microscopy plays an important role in the initial identification of obligate anaerobes in oro-dental infections, allowing for rapid visualization of bacteria and their morphological characteristics.

Techniques for Direct Microscopy:

1. **Gram Staining**: Gram staining is a primary step in direct microscopy, allowing for the differentiation of bacteria based on their cell wall properties. Obligate anaerobes, such as *Porphyromonas gingivalis* and *Prevotella intermedia,* are typically gram-negative rods, while *Peptostreptococcus* species are gram-positive cocci. The polymicrobial nature of many oro-dental infections can often be visualized with both gram-positive and gram-negative organisms seen in the same sample.

2. **Dark-Field Microscopy**: This technique can be used to visualize motile anaerobes, such as *Treponema denticola* and other spirochetes, which are implicated in periodontal diseases. Dark-field microscopy is particularly useful when assessing the motility and morphology of spiral-shaped bacteria that are difficult to detect with standard staining methods.

3. **Phase-Contrast Microscopy**: This technique enables the observation of live bacterial cells without staining. This method is especially useful for detecting *Treponema* species in subgingival plaque samples from periodontal pockets.

4. **Fluorescence Microscopy**: Fluorescent stains, such as acridine orange or ethidium bromide are used. These methods are useful for rapid visualization in mixed infections but may require specific antibodies or probes to identify obligate anaerobes at a species level.

ANAEROBIC CULTURE

Anaerobic culture media: [126 - 128]

- Non-selective media: Brucella Blood agar enriched with hemin and vitamin K, Robertson's cooked meat media and Thioglycolate broth (to support the growth of both anaerobic and facultative organisms).

- Selective media: Neomycin Blood Agar, Kanamycin-Vancomycin laked blood agar (to isolate anaerobic Gram-negative bacteria) or Phenylethyl alcohol (PEA) agar (inhibits gram-negative aerobes and allows anaerobes to grow).

Anaerobic culture techniques:

These are used to grow and isolate anaerobic microorganisms

a. McIntosh and Fildes' anaerobic jar :

- Anaerobic jars are used for cultivating anaerobic bacteria on plated media by creating an oxygen-free environment. This is typically achieved by introducing a gas mixture containing hydrogen (H2), which reacts with oxygen in the presence of a palladium catalyst to form water, establishing anaerobiosis. Indicators like methylene blue or resazurin are used to confirm the anaerobic conditions. Gas-generating envelopes or catalyst-free systems can also be used to create anaerobic conditions. Alternatively, the evacuation-replacement method removes air and replaces it with a nitrogen-hydrogen-carbon dioxide gas mixture for faster anaerobiosis.[54]

b. Anaerobic gas pouch

- Commercial anaerobic bag systems include the GasPak Pouch (BBL Microbiology Systems), AnaeroPouch (Mitsubishi), AnaerogenCompact (Oxoid), Anabag (Hardy

Diagnostics), and Anaerocult P (Merck). The GasPak Pouch features a clear, sealed bag, a catalyst-free liquid reagent for creating an anaerobic atmosphere, and a methylene blue indicator. Anaerocult P uses rusting iron filings to eliminate oxygen, while AnaeroPouch and Anaerogen activate on contact with air, requiring no additional water or reagents. The Anabag is large enough to accommodate a 150 mm plate for susceptibility testing or multiple 100 mm plates.

c. Anoxomat

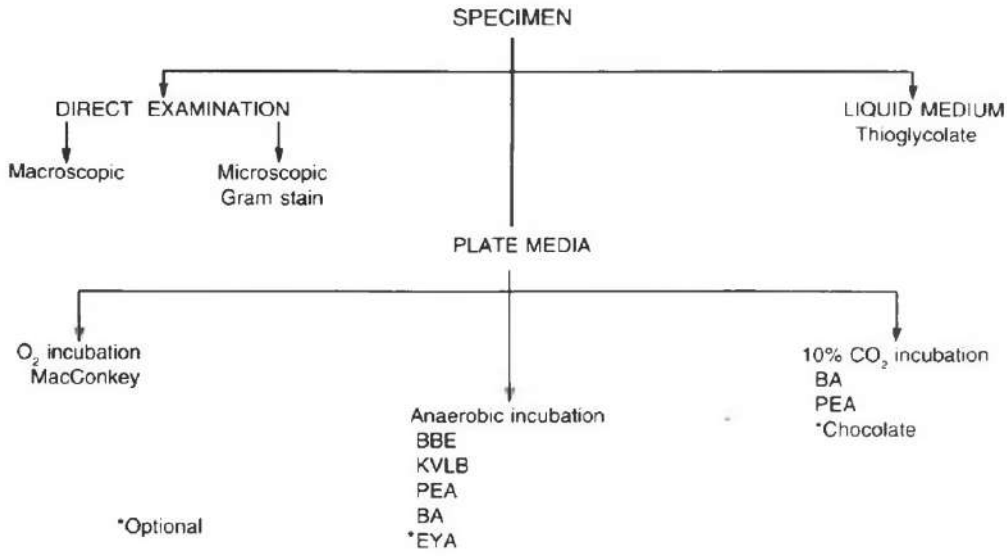
- The Anoxomat is an automated system that uses the evacuation-replacement method to create an anaerobic environment in jars. This system includes a built-in quality assurance program that performs tests for leak detection, catalyst activity, and proper gas replacement. Studies comparing the Anoxomat with conventional anaerobic jars and chambers have shown a slight improvement in recovering anaerobes, particularly from clinical specimens like those from the oral cavity, when using the Anoxomat system.

d. Anaerobic gas chamber

- Anaerobic chambers can be made from either flexible plastic or rigid, gas-tight cabinets. In the glove-sealed chambers (e.g., from Coy Laboratory Products or Forma Scientific), microbiologists access the chamber through gloves attached to it, though the gloves may reduce dexterity. Glove-free chambers (e.g., from Sheldon Manufacturing, Ruskinn Technology, and Don Whitley) use cuffed sleeves that seal around the forearm, allowing easier hand movement after evacuating the sleeve's air with a foot pedal. Both systems use an interchange to transfer materials in and out of the chamber, but glove-free systems allow small items to pass directly through the sleeve.To maintain an anaerobic environment, the chamber atmosphere is replaced with a gas mix of 5% hydrogen, 5-10% carbon dioxide, and 85-90% nitrogen, with palladium catalysts used to eliminate any oxygen leaks. Regular use requires rejuvenating the catalysts and changing moisture-absorbing desiccants. Methylene blue or resazurin indicators monitor oxygen levels, while the addition of Anatox, a charcoal-like substance, helps protect catalysts from contamination by volatile substances produced by anaerobes.

- **Incubation:** 35–37°C for 48–96 hours, but some cultures require longer incubation (up to 5–7 days)

Bacterial identification:



Aerotolerance testing [54]

- 2 plates of brucella blood agar were inoculated with the test organism and control organisms (Pseudomonas aeruginosa ATCC 10145 and Bacteroides fragilis ATCC 25285).
- One plate was incubated at 37°C under aerobic conditions for 24 hours and the second one was incubated at 37°C in anaerobic jar for 48 hours.

<u>Conventional method of bacterial identification</u> [54, 126-128]

Biochemical identification

a. Catalase Test
b. Indole Production Test
c. Nitrate Reduction Test
d. Bile Sensitivity Test
e. Esculin Hydrolysis Test
f. Fermentation of Sugars

<u>Automation Methods</u>

1. Automation systems based on biochemical identification [134,135]
   a. VITEK-2 Compact (bioMérieux, USA)

b. BD Phoenix (Becton, Dickinson and Company, USA)

2. Matrix-Assisted Laser Desorption Ionization–Time of Flight mass spectrometry (MALDI-TOF MS) [136-138]

Automation systems based on biochemical identification

a. VITEK-2 Compact (bioMérieux, USA)

b. BD Phoenix (Becton, Dickinson and Company, USA)

Principle:

The systems operates on the principle of automated biochemical testing:

1. **Sample Preparation**: Pure cultures of anaerobic bacteria are prepared from appropriate culture media.
2. **Inoculation**: The bacterial suspension is inoculated into an identification card containing wells with various biochemical substrates.
3. **Incubation**: The inoculated card is placed in an incubator, where it is maintained under optimal growth conditions.
4. **Detection**: The system detects changes in color in the wells, indicating biochemical activity, and records these changes over time.
5. **Identification**: The data is analyzed by proprietary software that compares the growth profile to a comprehensive database of known microorganisms.

Advantages:

- High throughput and quick turnaround time.
- Automated reading reduces human error.

Limitations:

- The accuracy can be influenced by the quality of the database and the performance of the tests.

Steps of identification:

- Isolation: Grow the anaerobic bacteria on suitable media for 48-96 hours.
- Inoculum Preparation: Prepare a bacterial suspension to a 0.5 McFarland standard.

- Inoculation: Inoculate the suspension into a identification card.
- Incubation: Place the card in the incubator and incubate for the required duration.
- Monitoring: The system automatically monitors and detects changes in color.
- Data Analysis: After incubation, the results are analyzed by the software, which provides identification.

Quality Control: Regularly perform quality control checks with known bacterial strains.

Matrix-Assisted Laser Desorption Ionization–Time of Flight mass spectrometry (MALDI-TOF MS) [134-136]

Principle:

The principle behind MALDI-TOF involves the following steps:

1. **Sample Preparation:** Bacterial colonies are mixed with a matrix solution containing small organic molecules (usually a-cyano-4-hydroxycinnamic acid or sinapinic acid) that absorb UV light.

2. **Desorption and Ionization:** The matrix is applied to a target plate, and a laser is directed onto the sample. The laser energy causes the matrix to evaporate rapidly, leading to the desorption and ionization of the bacterial proteins.

3. **Time-of-Flight Analysis:** The ionized proteins are accelerated in an electric field and travel through a vacuum tube to a detector. The time taken for the ions to reach the detector (time-of-flight) is measured, and this time is directly related to their mass-to-charge ratio (m/z).

4. **Spectrum Generation:** The result is a mass spectrum displaying peaks that correspond to different proteins or fragments of proteins present in the bacterial sample. Each peak's intensity and position create a unique protein fingerprint specific to the bacterial species.

5. **Identification:** The generated mass spectrum is compared to a reference database containing spectra of known bacterial species. Using software algorithms, the closest match is identified, providing rapid and accurate species identification.

Systems Available in India:

1. **Bruker MALDI Biotyper**
   - Features: This system is widely used for clinical diagnostics. It offers a large database for bacteria and fungi identification, including anaerobes. The Biotyper provides rapid identification and has a user-friendly interface.
   - Application: Suitable for routine identification of anaerobic bacteria from clinical samples, including oro-dental isolates.

2. **Vitek MS Prime (bioMérieux)**
   - Features: The Vitek MS Prime is designed for fast and reliable identification of microorganisms, including anaerobes. It features an extensive database and automated processes, enhancing the speed of identification.
   - Application: Particularly suitable for clinical laboratories focusing on microbiological diagnostics, including oro-dental infections

3. **Abbott ID NOW™**
   - Features: This compact and portable MALDI-TOF system can rapidly identify pathogens, including anaerobic bacteria, in a range of clinical samples. It is known for its speed and efficiency.
   - Application: Useful in point-of-care testing settings for rapid identification.

4. **Shimadzu MALDI-8020**
   - Features: This instrument provides high sensitivity and resolution for protein analysis. It is equipped with advanced software for spectral analysis and identification.
   - Application: Applicable for research and clinical laboratories focusing on anaerobic bacterial identification.

5. **Thermo Scientific™ LTQ Orbitrap™**
    - Features: This high-resolution mass spectrometer is designed for complex mixtures and provides high sensitivity. While it is more commonly used for proteomics, it can identify anaerobic bacteria when configured properly.
    - Application: Suitable for in-depth analysis of anaerobic pathogens.

6. **Waters Xevo G2-XS QToF**
    - Features: This is a high-resolution mass spectrometry system that can perform both qualitative and quantitative analyses. It is capable of detecting a wide range of microbial species.
    - Application: Effective for detailed studies on anaerobic bacterial identification.

Advantages:

- Rapid Identification
- High Accuracy and Specificity
- Cost-Effective in the Long Term
- Minimal Sample Preparation
- Broad Applicability

Limitations:

- Limited to Known Organisms in the Database
- Initial Cost
- Requirement of Pure Cultures
- Expertise

<u>Steps of identification in MALDI-TOF</u>:

1. **Isolation of Bacterial Colonies:**
    - Culture obligate anaerobes on appropriate anaerobic media
    - Incubate under anaerobic conditions (e.g., anaerobic chamber or jar) for 48-96 hours.

2. **Preparation of Samples:**
    - Select well-isolated colonies that exhibit typical morphology of the suspected anaerobes.

- Use a sterile inoculating loop to pick a small amount of the colony and place it on the target plate.
- Add 1-2 µL of the matrix solution to the same spot on the target plate and mix gently to create a homogenous mixture.

3. Drying

- Allow the sample to dry at room temperature in a sterile environment.

4. MALDI-TOF Analysis:

- Insert the target plate into the MALDI-TOF mass spectrometer.
- Set the parameters according to the manufacturer's instructions, ensuring the instrument is calibrated for optimal performance.
- Acquire mass spectra for each sample, which typically takes a few minutes.

5. Data Interpretation:

- Use software associated with the MALDI-TOF instrument to compare the acquired spectra with the reference database.
- Identify the bacterial species based on the best match with the database entries.

Quality Control:

- Include quality control strains with known identities to ensure the accuracy and reliability of the results.

Identification capabilities of anaerobic organisms by different microbial identification systems. [139-141]

Automated System	Number of Identifiable Anaerobic Organisms
MALDI-TOF MS Prime	~3,000 species
Bruker MALDI-TOF	>700 species
Vitek 2 Compact	~600 species
BD Phoenix	~500 species

Molecular Methods [142-146]

1. Nucleic Acid Hybridization Techniques
2. Conventional PCR
3. Real-Time PCR (qPCR)
4. Next-Generation Sequencing (NGS)

1. Nucleic Acid Hybridization Techniques [142]

Description: These techniques involve the use of labeled DNA probes that hybridize to specific target sequences in the DNA of obligate anaerobes.

- **Application**: Techniques such as Fluorescent In Situ Hybridization (FISH) can be used to visualize specific anaerobic bacteria in biofilms or dental plaque.

- **Advantages**:
    - Allows for direct visualization of bacteria in the sample matrix.
    - Can be used to detect multiple species simultaneously.

- **Limitations**:
    - Requires specific probes for each target organism.
    - Often labor-intensive and requires sophisticated imaging systems.

2. Conventional PCR [143,144]

Description: PCR is a widely used technique that amplifies specific DNA sequences from microbial cells. By targeting unique genes or ribosomal RNA (rRNA) genes, PCR can detect and identify specific obligate anaerobes.

- **Application**: PCR is used to identify species such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Prevotella intermedia* in clinical samples.

- **Advantages**:
    - High sensitivity and specificity.

- Ability to detect non-culturable and fastidious organisms.

- **Limitations**:
  - Requires knowledge of specific target sequences.
  - Possible contamination leading to false positives.

3. Real-Time PCR (qPCR) [145]

Description: qPCR allows for the quantitative measurement of DNA, enabling not just identification but also estimation of bacterial load in samples.

- **Application**: Often used to quantify pathogenic anaerobes, such as *Bacteroides fragilis* and *Treponema denticola*, in periodontal and endodontic infections.

- **Advantages**:
  - Rapid results and quantification.
  - Higher sensitivity compared to traditional PCR

- **Limitations**:
  - Requires expensive equipment and reagents.
  - Complexity in data interpretation.

4. Next-Generation Sequencing (NGS) [146]

Description: NGS allows for comprehensive profiling of microbial communities, providing insights into the diversity and composition of obligate anaerobes present in oro-dental infections.

- **Application**: Used for metagenomic studies to understand the microbial ecology of periodontal disease and root canal infections.

- **Advantages**:
  - High-throughput capability.
  - Provides a comprehensive view of the microbiome.

- **Limitations**:
    - Requires bioinformatics expertise for data analysis.
    - Higher cost compared to other molecular techniques.

LABORATORY DETECTION METHODS OF ANTIMICROBIAL RESISTANCE

[147]

The antimicrobial susceptibility of anaerobic organisms was determined by measuring the Minimum Inhibitory Concentration (MIC) values, in accordance with the guidelines provided by CLSI and EUCAST. [148,149]

Methods or technologies used in the AMR detection: [147]

A. Phenotypic methods

1. Dilution Methods

2. Gradient testing (E-Strip Method)

3. Spiral gradient endpoint system

B. Automation method by sensititre

C. PCR-Based methods for detection of resistance genes

A. Phenotypic methods

1. Dilution Methods

Agar Dilution [150]

The agar dilution method is a recommended AST technique for testing many different anaerobic organisms as per the CLSI guidelines.[148] MIC is defined as the lowest drug concentration inhibiting visible bacterial growth in agar or broth, typically expressed in mg/L (μg/mL). This method remains widely used today for quantitative AST, particularly for new drug development. In agar dilution, the antimicrobial agent is serially diluted two-fold, mixed with molten agar, and solidified. A 0.5 McFarland turbidity standard microbial inoculum is then applied, and the plate is incubated overnight at 35-37°C. The MIC is determined by identifying the agar plate with the lowest antibiotic concentration showing no bacterial growth, evidenced by colony-forming units (CFU). While traditionally, a single organism and antibiotic

concentration are tested per plate, inoculum replicators enable simultaneous testing of multiple organisms (32-36 inocula per plate). The agar dilution method offers simplicity, known parameters, and cost-effectiveness when using replicators. However, it is labor-intensive process with prolonged turnaround times.

Broth dilution [150]

There are two primary types of dilution methods: microdilution and macrodilution. The broth microdilution method is a recommended AST technique for testing *Bacterioides spp* and *Parabacterioides spp* as per CLSI guidelines.[148] Both utilize broth or agar as the medium for bacterial growth. In broth dilution, antibiotics are subjected to consecutive two-fold dilutions, typically in increments of 1, 2, 4, 8, and 12 µL. These dilutions are then dispensed into micro-centrifuge tubes containing bacterial growth medium, and the final volume is achieved by adding medium. After overnight incubation at 35 °C, growth is examined to determine the breakpoint based on turbidity of the culture media. Dilution methods have several significant limitations that hinder their effectiveness. Foremost among these is the requirement of large volumes of reagents, which can be cumbersome and costly. Experimental space constraints can limit the number of tests that can be conducted simultaneously. The tedious dilution steps involved in macrodilution methods can lead to human error. Furthermore, prolonged incubation times increase the risk of false positive results.

Benefits:

- Well-defined parameters
- Cost-effective with inoculum replicators

Limitations:

- Required Training
- Labor-intensive
- Long turnaround times

Other potential drawbacks of dilution methods include:

- Cross-contamination risks

To achieve reliable results, maintaining optimal testing parameters is crucial. This includes:

- Precise pH control
- Strict temperature regulation
- Selection of suitable media
- Adherence to recommended incubation times

Moreover, conducting a control viability plate is mandatory to ensure practical clinical relevance. These limitations underscore the need for careful consideration and optimization of dilution methods to ensure accurate and reliable antimicrobial susceptibility testing.

2. E-Strip Method [150]

The Epsilometer test (Etest) represents a significant advancement in routine analysis of antibiotic resistance in bacteria. Developed by Bolmström and Eriksson in the late 1980s, this method gained prominence with the introduction of the first Etest plastic strip by AB BIODISK in 1991.The Etest strip is coated with predefined antibiotic concentrations, with corresponding MIC ranges marked on the surface and back. To perform the test, multiple strips are placed on a pre-inoculated agar plate, followed by overnight incubation. The resulting elliptical inhibition zones around the strips indicate the MIC at the intersection point between the zone and strip edge.The Etest's simplicity, accuracy, and reliability have made it an attractive option for clinical laboratories, leading to FDA-approved commercialization. Its convenience and flexibility in interpreting MIC values under various physical conditions have rendered it a preferred method over traditional dilution techniques for antimicrobial susceptibility testing (AST).

Benefits :

- Simultaneous testing of multiple antibiotics
- Pre-defined antibiotic concentrations
- Clear MIC interpretation
- Adaptability to diverse physical conditions
- Accuracy and reliability

Limitations :

- Inconsistent and inaccurate performance for certain antibacterial agents, including Penicillin, Ciprofloxacin, Ofloxacin, and Rifampicin.
- High costs, stringent storage requirements for Etest strips, and laboratory setup demands for optimal plate inoculation and incubation conditions can strain resources.

3. Spiral Gradient Endpoint System [151]

The Spiral Gradient Endpoint (SGE) method is a specialized technique often used in clinical microbiology to determine the minimum inhibitory concentration (MIC) of antimicrobial agents, particularly for anaerobic bacteria.

- **Spiral Plating**: The technique involves a spiral plater that deposits a gradient of antimicrobial agents onto the surface of an agar plate. The concentration of the antibiotic gradually decreases from the center of the plate outward.
- **Inoculation with Anaerobes**: The test organism (anaerobic bacteria) is inoculated onto the plate, where it will grow in areas where the concentration of the antibiotic is too low to inhibit growth.
- **Gradient System**: Since the antimicrobial agent's concentration decreases in a radial fashion, the method provides a continuous gradient, offering a more precise determination of the MIC than traditional stepwise methods.
- **Endpoint Determination**: The endpoint is identified as the point where bacterial growth is inhibited along the gradient. This allows for the calculation of the MIC, which corresponds to the concentration of the drug at the point of growth inhibition.

Benefits:

- Continuous gradient system
- Precise MIC determination
- Efficient and accurate

Limitations:

- The SGE method requires specific, often costly, spiral platers

- Limited range of antibiotics
- Subjectivity in endpoint reading

B. Automation methods by sensititre: [151]

ThermoFisher Scientific offers two ready-made panels for anaerobic antimicrobial susceptibility testing through its Sensititre brand. The AN02B dried panel includes 15 antimicrobials with varying dilution ranges and has a 1-2 year shelf life at room temperature. These panels are designated for research use only.

Benefits:

1. Convenience: Pre-formulated panels save time and labor.
2. Standardization: Consistent, quality-controlled panels ensure reproducible results.
3. Broad antimicrobial coverage: Multiple agents tested simultaneously.
4. Easy interpretation: Clear, visual results facilitate interpretation.
5. Reduced errors: Minimized risk of human error in preparing test solutions.

Limitations:

1. Limited flexibility: Pre-defined panels may not accommodate specific research needs.
2. Research-use only: Not intended for diagnostic testing.
3. Stability concerns: Panels may degrade over time, affecting accuracy.
4. Limited anaerobe coverage: May not include all relevant anaerobic bacteria.
5. Quality control: Dependence on manufacturer's quality control measure

C. PCR-based methods for detection of resistance genes: [151]

PCR-based molecular assays are commonly utilized to identify genes responsible for antibiotic resistance, specifically nim genes (metronidazole resistance) and cfiA-type genes (carbapenem resistance).

Benefits

- Rapid and can identify known resistance genes directly.

Limitations

- Limited to known resistance genes (misses' novel or unknown mechanisms).

Used in clinical and research settings to detect known resistance mechanisms like *cfiA* for carbapenem resistance in *Bacteroides*.

OBJECTIVES

OBJECTIVES

1. To analyse the distribution of obligate anaerobes causing orodental infections.
2. To analyse the antimicrobial resistance pattern of obligate anaerobic isolates obtained from cases of orodental infections.
3. To compare the results of antimicrobial susceptibility testing of E-strip method with the gold standard Agar dilution method.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study was initiated after obtaining ethical clearance from Institutional Human Ethics Committee - Student Research. (AIIMS/BPL/IHECSR/July/22/PG/09) dated 28th February 2023.

1. STUDY DESIGN

Observational cross-sectional study

2. STUDY SETTING

The study was carried out in the Departments of Microbiology and Dentistry at All India Institute of Medical Sciences (AIIMS), Bhopal.

3. STUDY DURATION

March 2023 to September 2024 (1 year and 6 months)

4. STUDY POPULATION

4.1. Sample size: 200

The study population was subdivided into two groups of 100 each as follows

I. Patients suffering from Dental caries (n=100)
II. Patients suffering from Periodontitis (n=100)

4.2. Case definition

Dental caries:

Patient presented with the localized demineralization and degradation of the hard tissues of the teeth, resulting in the progressive destruction of tooth structure.[2]

Periodontitis:

Patient presented with inflammation and damage to the periodontal tissues, leading to loss of alveolar bone support and potential tooth loss.[27]

4.3.Inclusion Criteria

1. Patients suffering from dental caries and periodontitis visiting to dental OPD/dental camps organized by the institute.
2. Patients above 5 years of age.

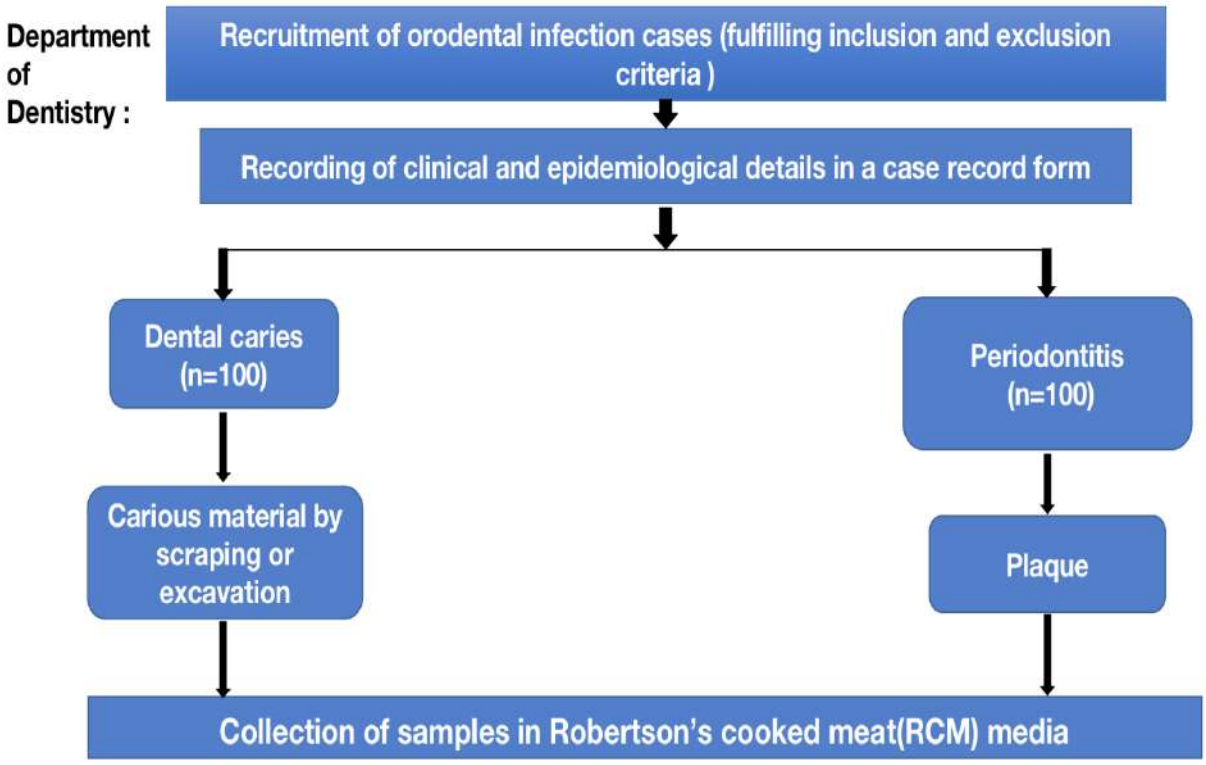
4.4. Exclusion Criteria

1. Patients on antibiotic treatment for the past 5 days.
2. Patients with restricted mouth opening.
3. Patients who have undergone recent oral surgeries.

   Patients not willing to participate in the study.

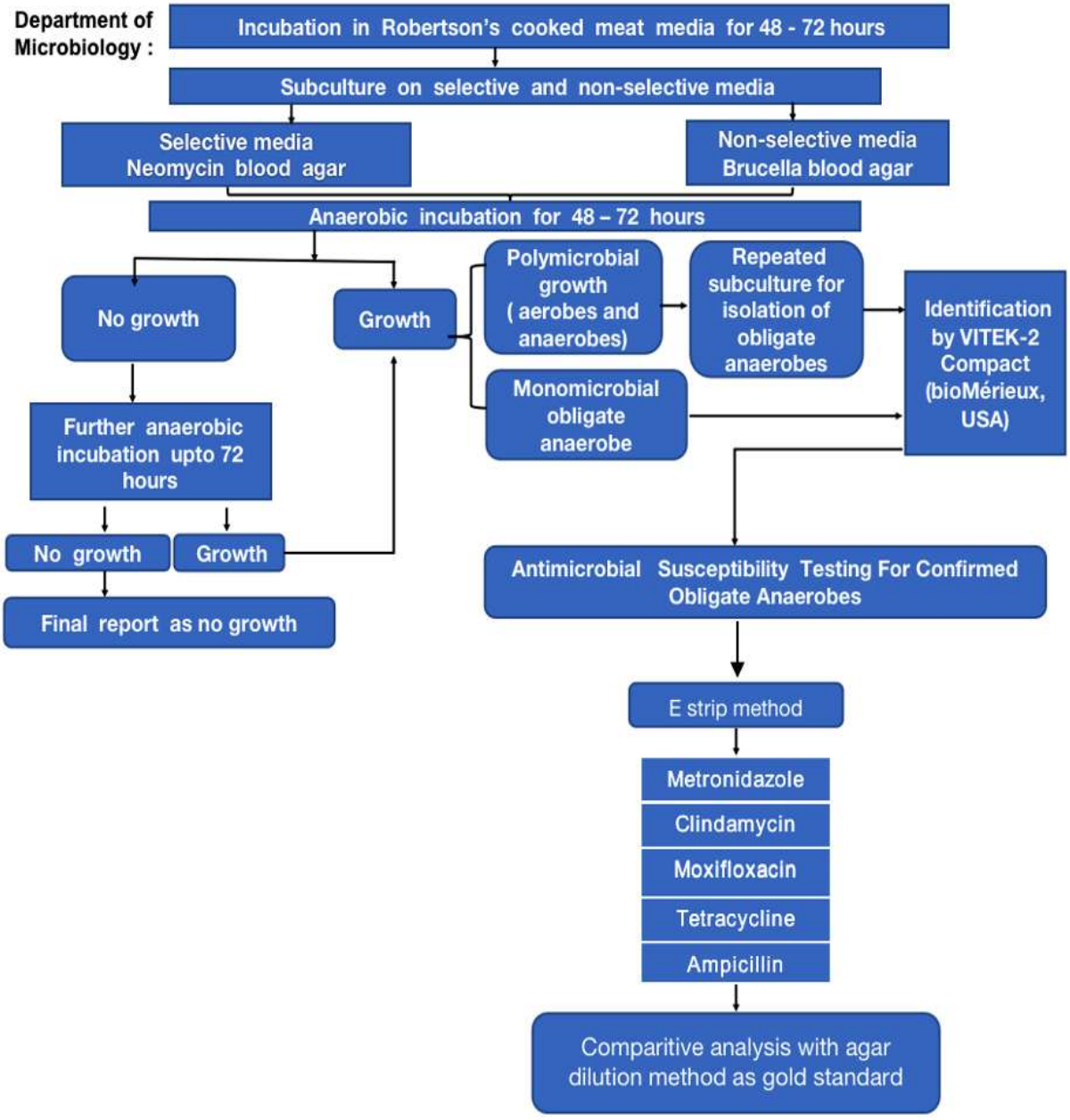
5. SPECIMEN COLLECTION AND TRANSPORTATION [152,153,154] :

After identifying the patient, a detailed clinical history and associated risk factors were recorded in a pre-designed case record form. (Annexure 1)


Department of Dentistry :
Recruitment of orodental infection cases (fulfilling inclusion and exclusion criteria)
Recording of clinical and epidemiological details in a case record form
Dental caries (n=100)
Periodontitis (n=100)
Carious material by scraping or excavation
Plaque
Collection of samples in Robertson's cooked meat(RCM) media

Sr.No.	Clinical conditions	Type of specimen collected	Transportation conditions
1	Dental caries	Carious material by scraping / excavation	In Robertson's cooked meat(RCM) media as soon as possible; In case of delay ,samples to be kept at room temperature or incubators
2	Periodontitis	Plaque	

6. SAMPLE PROCESSING


Department of Microbiology :
Incubation in Robertson's cooked meat media for 48 - 72 hours
Subculture on selective and non-selective media
Selective media Neomycin blood agar
Non-selective media Brucella blood agar
Anaerobic incubation for 48 – 72 hours
No growth
Growth
Polymicrobial growth (aerobes and anaerobes)
Repeated subculture for isolation of obligate anaerobes
Identification by VITEK-2 Compact (bioMérieux, USA)
Monomicrobial obligate anaerobe
Further anaerobic incubation upto 72 hours
No growth
Growth
Final report as no growth
Antimicrobial Susceptibility Testing For Confirmed Obligate Anaerobes
E strip method
Metronidazole
Clindamycin
Moxifloxacin
Tetracycline
Ampicillin
Comparitive analysis with agar dilution method as gold standard

Isolation of anaerobes

6.1. Bacterial culture

- Specimens collected in Robertson cooked meat media are sub-cultured along with metronidazole disc (5μg) onto

    a. Non selective media: Brucella blood agar
    b. Selective media: Neomycin blood agar

- The plates are further incubated for 48 - 72 hours under anaerobic conditions in anaerobic jar with gas pack (HiMedia, Mumbai) / Anoxomat (Don Whitley Scientific Limited, U.K)

- Culture plates are observed after 48 hours for the presence of growth.

- **Quality Control**

  Biological indicators are used

  a) Anaerobic indicator - *B.fragilis* ATCC 25285

  b) Aerobic indicator - *Pseudomonas aeruginosa* ATCC 10145

6.2. Identification of isolate [54]

The primary culture plates i.e. Brucella blood agar and Neomycin blood agar were first examined and colony characteristics were noted. A single well separated colony was identified by the following methods.

6.2.1. Gram Staining (Kopeloff – Beerman's modification) [54]

- The suspected colony was picked up with a loop and emulsified in sterile normal saline on a glass slide.
- One smear for positive and negative control was also put up on the same glass slide.
- The smears were allowed to dry and then fixed with absolute methanol and allowed to dry.
- The methanol fixed smear was flooded with 1% crystal violet and 5 drops of 5% sodium bicarbonate was added. It was allowed to stand for 1 minute and excess stain was washed off with tap water.
- The smear was then flooded with Gram's iodine for 1 minute and then washed under tap water.

- Decolourisation was done with Acetone Alcohol (3:7 ratio) for 5 seconds and immediately washed under tap water.
- The smear was then flooded with 1% aqueous safranine and allowed to stand for 2 minutes.
- Excess stain was washed off and the slide was allowed to dry completely before visualising under 100x objective (Oil Immersion)
- **Interpretation:** Either Gram positive cocci/bacilli or Gram negative cocci/bacilli.
- **Quality Control**
  a) Gram positive control - *Clostridium perfringens* ATCC 13124
  b) Gram negative control - *Bacteroides fragilis ATCC 25285*

6.2.2. Aerotolerance test [54]

- 2 plates of brucella blood agar were inoculated with the test organism and control organisms
- One plate was incubated at 37°C under aerobic conditions for 24 hours and the second one was incubated at 37°C in anaerobic jar for 48 hours.
- **Quality Control**
  a) Anaerobic indicator - *B.fragilis* ATCC 25285
  b) Aerobic indicator - *Pseudomonas aeruginosa* ATCC 10145

6.2.3. Identification by VITEK 2 Compact, (bioMerieux, USA)

Identification was done strictly as per the manufacturer's protocol

1. Identification of the isolate was done using the VITEK 2 ANC card as follows:
2. The VITEK 2 ANC card was allowed to reach the room temperature before use.
3. The inoculum suspension was prepared as follows:

- 3 ml of sterile saline was aseptically transferred into sterile clear polystyrene tube.
- Several isolated colonies were mixed in the normal saline using sterile metal loop to prepare a homogenous suspension.

4. Measurement of Turbidity:

- The instrument was turned on and set of plastic tube setting
- Blank was tested by inserting a test tube filled with sterile normal saline.

- The tube containing suspension of the organism was placed inside the instrument and slowly rotated.
- The turbidity was measured and adjusted to a value between 2.70 and 3.30 McFarland.

5. The tube is inserted in the cassette and the straw (in the ANC card) was inserted into inoculated suspension tube in the cassette.
6. Patient details were entered into the system as per the manufacturer's instructions.
7. The cassette was placed in the filler box and start fill button was pressed.
8. After the filling cycle was complete, the cards are removed and inserted into the load door.
9. The cassette was then removed and discarded into the biohazard bin.
10. The suspension was subcultured onto BBA to check for purity.
11. **Interpretation –** Identification upto the species level was provided by VITEK 2 compact.

6.2.4. Antimicrobial susceptibility testing by E-strip method

AST was performed for the following Antimicrobials:

1. Metronidazole
2. Clindamycin
3. Moxifloxacin
4. Tetracycline
5. Ampicillin

Procedure:

Inoculum Preparation [155]

The test inoculum was prepared by emulsifying 3-4 individual colonies of the test strain in saline. The turbidity of the inoculum was then compared to the 0.5 McFarland standard. If necessary, the turbidity of the inoculum was adjusted to match the standard, ensuring the correct microbial concentration for further analysis.

Inoculation on Brucella Blood Agar

1. A sterile cotton swab was dipped into the inoculum and then rotated against the inner wall of the tube to remove excess liquid.

2. The swab was streaked evenly across the agar surface, with the plate rotated 60 degrees to ensure uniform coverage.
3. The inoculation process was completed by running the swab around the rim of the plate.
4. The plate was then left with the lid on for 5 minutes to allow excess moisture to be absorbed before applying the strip.

Application of E-test strips (HiMedia, Mumbai)

1. The E-test package was thawed at room temperature for at least 30 minutes before use.
2. The package was opened, and an E-test strip was removed using sterile forceps.
3. The strip was applied to the inoculated agar surface, with the 'E end' positioned at the plate's edge and the numeric scale facing upwards.
4. The plates were incubated under anaerobic conditions at 37°C for 48 hours.

Interpretation of Results

E-Test Reading and Interpretation:

After 48 hours of anaerobic incubation:

1. The strips were viewed from the top of the plate, and a symmetrical inhibition ellipse was observed.
2. The Minimum Inhibitory Concentration (MIC**)** was read at the intersection of the ellipse's lower edge and the test strip.
3. The MIC value was recorded at the point of complete growth inhibition.

Table 6: Interpretation of MIC Values: [156]

		Interpretative categories and MIC breakpoints µg/ml		
Sr. No.	**Name of antimicrobial agent**	**Susceptible**	**Intermediate**	**Resistant**
1	Metronidazole	≤ 8	16	≥ 32
2	Clindamycin	≤ 2	4	≥ 8
3	Moxifloxacin	≤ 2	4	≥ 8
4	Tetracycline	≤ 4	8	≥ 16
5	Ampicillin	≤ 0.5	1	≥ 2

- **Quality Control**
  c) Gram positive anaerobes - *Clostridium perfringens* ATCC 13124
  d) Gram negative anaerobes - *Bacteroides fragilis ATCC 25285*

6.2.5. Antimicrobial susceptibility testing by Agar dilution method [157 – 162]

Procedure

Table 7: Calculations of Different Antibiotics Standard Solutions:

S.No	Antibiotic	Solvent	Diluent	Calculation (Formula)
1.	Metronidazole	DMSO (Dimethyl sulfoxide)	Distilled Water	Potency = 95% Weight (mg) = 100 (ml) X 5120 (µg/ml)/950 (µg/ml) = 538.9ml 538.9ml = 0.53mg(converted ml to mg)
2.	Clindamycin	Distilled Water	Distilled Water	Potency = 96% Weight (mg) = 100 (ml) X 5120 (µg/ml)/960 (µg/ml) = 533.3ml

				533.3ml = 0.53mg(converted ml to mg)
3.	Moxifloxacin	Distilled Water	Distilled Water	Potency = 98% Weight (mg) = 100 (ml) X 5120 (µg/ml)/980 (µg/ml) = 522.44ml 522.4ml = 0.52mg(converted ml to mg)
4.	Tetracycline	Distilled Water	Distilled Water	Potency = 90% Weight (mg) = 100 (ml) X 5120 (µg/ml)/900 (µg/ml) = 568.88ml 568.8ml = 0.56mg(converted ml to mg)
5.	Ampicillin	0.1 M Phosphate buffer, pH 6	0.1 M Phosphate buffer, pH 6	Potency = 96% Weight (mg) = 100 (ml) X 5120 (µg/ml)/900 (µg/ml) = 533.33ml 533.3ml = 0.53mg(converted ml to mg)

Preparation of antimicrobial stock solution and dilution

Scheme for preparing dilutions of antimicrobial agents to be used in agar dilution test.[54]

	Antimicrobial Solution						
Step	Concentration (μg/mL)	Source	Volume (mL)	Diluent (mL)	Intermediate Concentration (μg/mL)	Final Concentration at 1:10 Dilution in Agar (μg/mL)	Log_2
	5120	Stock	–	–	5120	512	9
1	5120	Stock	2	2	2560	256	8
2	5120	Stock	1	3	1280	128	7
3	5120	Stock	1	7	640	64	6
4	640	Step 3	2	2	320	32	5
5	640	Step 3	1	3	160	16	4
6	640	Step 3	1	7	80	8	3
7	80	Step 6	2	2	40	4	2
8	80	Step 6	1	3	20	2	1
9	80	Step 6	1	7	10	1	0
10	10	Step 9	2	2	5	0.5	−1
11	10	Step 9	1	3	2.5	0.25	−2
12	10	Step 9	1	7	1.25	0.125	−3

NOTE: This table is modified from Ericsson HM, Sherris JC. Antibiotic sensitivity testing. Report of an international collaborative study. *Acta Pathol Microbiol Scand.* 1971;217(suppl B):1-98.

S.No	Antimicrobial agent	Dilutions made
1.	Metronidazole	5 doubling dilutions (4-64 μg/ml)
2.	Clindamycin	5 doubling dilutions (1-16μg/ml)
3.	Moxifloxacin	5 doubling dilutions (1-16μg/ml)
4.	Tetracycline	5 doubling dilutions (2-32μg/ml)
5.	Ampicillin	5 doubling dilutions (0.25-4μg/ml)

For the preparation of antibiotic dilutions using the agar dilution method, I selected from the concentration that is one MIC lower than the sensitivity range till the concentration that is one MIC higher than the resistance range of the specific antimicrobials to ensure that the full range of susceptibility can be assessed.[156]

Dilutions of Antimicrobials

Preparation of plates:

Media used – Brucella Blood Agar + Vit K1 + Hemin

Add 17ml of agar base + 1ml of laked blood + 2ml diluted antibiotic solution in a Mc Cartney bottle

↓

Mixed well by gently inverting and pouring in a sterile petri plate

↓

Kept the plate in a sterile place till it get solidified

Preparation of inoculum (Direct colony suspension method)

Suspended colonies from 24-48 hrs blood culture directly into broth

↓

Compared it with 0.5 mc farland using wickerham card

↓

Incubated anaerobically for half an hour

Inoculation of test isolates on the plates

Spot inoculated the test organism on the plate

↓

Left at room temperature for 10 mins for absorption and incubated under anaerobic conditions

↓

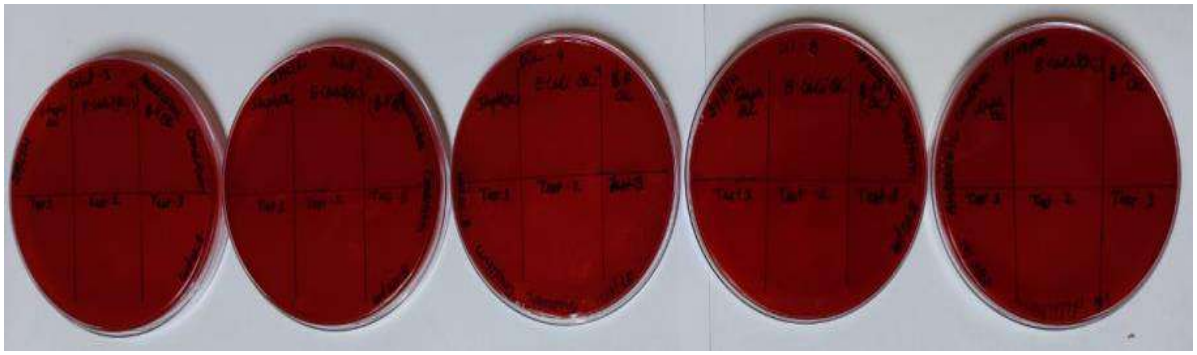
After 48 hours of anaerobic incubation readings were taken from the lowest concentration to the highest concentration

↓

Reading was taken as: No Growth
Haze
1 Discrete colony
Multiple tiny colonies

17ml agar base + 1ml Laked blood + 2ml dilution antibiotic solution

↓



Leave the plate for 10 min for absorption of inoculum

Controls for antimicrobial susceptibility testing

1. *Bacteroides fragilis ATCC 25285*
2. *Clostridium difficile ATCC 700057*

QC sterility check [148,149]

According to CLSI (Clinical and Laboratory Standards Institute) guidelines, agar dilution sterility checks should be performed with each batch of agar prepared.[156,158]

1. **During Plate Preparation:**
    - Empty Control Plate: Prepare a blank agar plate without any inoculation or antimicrobial agents to ensure that the prepared media is sterile.
    - Incorporate During Pouring: While pouring the plates with antimicrobial dilutions, include a plate that contains only the agar medium (no antimicrobials) to check for sterility of the medium and the preparation process.
    - Incubation: Incubate the blank control plates under the same conditions as the test plates to confirm the sterility of the media and ensure no bacterial growth occurs.
2. **During Inoculation:**
    - Uninoculated Plate: Alongside the test plates, include an uninoculated control plate (with antimicrobial agent but without bacteria). This checks for contamination during the inoculation process (e.g., handling or environmental contamination).
    - After inoculating the test organisms, place the control plate in the incubator alongside the inoculated plates and monitor for growth.
3. **Post-Inoculation and During Incubation:**
    - Sterility Plate Post-Inoculation: After all plates have been inoculated, you may place another control plate to check for contamination introduced during the final stages of inoculation or placement in the incubator.
    - If this plate shows any growth, it indicates that contamination occurred during or after inoculation.
4. **After Incubation:**
    - Examine the control plates for bacterial growth.

- If any growth appears on the sterility control plates, it suggests contamination in the corresponding stage (e.g., during media preparation, inoculation, or incubation).

Interpretation of Sterility Check:

- No Growth: The process was sterile, and any growth on test plates is due to the test organism.
- Growth Detected: Indicates contamination at the stage where the plate was placed, requiring repeat testig and investigation of possible sources of contamination.

A **B** **C**

Image 1: Sterility check

A: Uninoculated control plate with antibiotic

B: Sterility plate post Inoculation

C: Sterility plate After Anaerobic incubation

Interpretation of results [156]

		Interpretative categories and MIC breakpoints µg/ml		
Sr. No.	**Name of antimicrobial agent**	**Susceptible**	**Intermediate**	**Resistant**
1	Metronidazole	≤ 8	16	≥ 32
2	Clindamycin	≤ 2	4	≥ 8
3	Moxifloxacin	≤ 2	4	≥ 8
4	Tetracycline	≤ 4	8	≥ 16
5	Ampicillin	≤ 0.5	1	≥ 2

7. DATA ANALYSIS

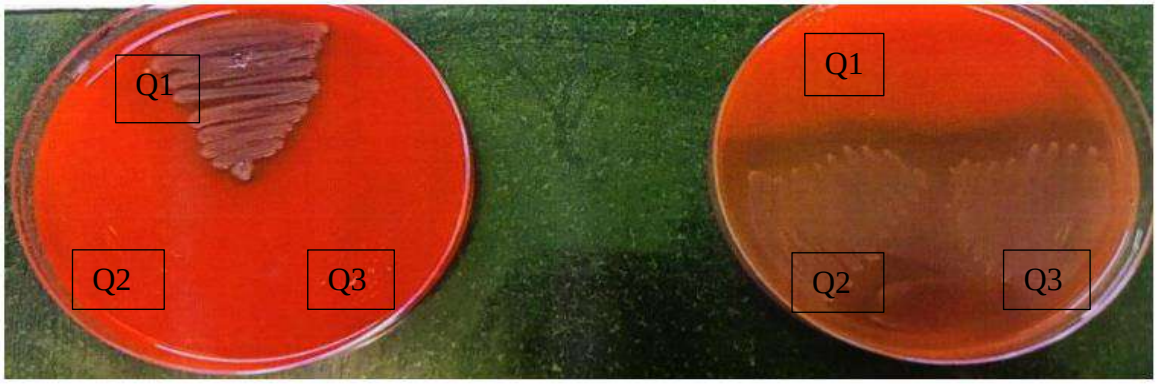
1. The collected data was entered into Microsoft Excel and then analyzed using SPSS version 25.
2. The normality of each variable was assessed using the Kolmogorov-Smirnov test.
3. Quantitative data was expressed as mean and standard deviation. The difference between the means of two groups was tested using the Mann-Whitney U test.
4. Qualitative data was expressed as percentages, and the difference between the percentages of two groups was tested using the Chi-square test or the Fisher's exact test, as appropriate.
5. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the E-strip method were calculated to assess the sensitivity pattern of different antibiotics.
6. A p-value of less than 0.05 was considered statistically significant.

RESULTS

RESULTS

Image 2: Anaerobic jar with gas pack

Image 3: Anoxomat , Jar gassing system (Don Whitley Scientific Limited, U.K)



Aerobic Plate	Anaerobic Plate
Quadrant 1 – P.aeruginosa ATCC 10145	Quadrant 1 – P.aeruginosa ATCC 10145
Quadrant 2 – B.fragilis ATCC 25285	Quadrant 2 – B.fragilis ATCC 25285
Quadrant 3 – Test organism	Quadrant 3 – Test organism

Image 4: Aerotolerance testing

Image 5: Growth of *Prevotella spp* on Brucella Blood Agar showing moist, circular, slightly convex, grey colour colonies with 1-2 mm diameter.

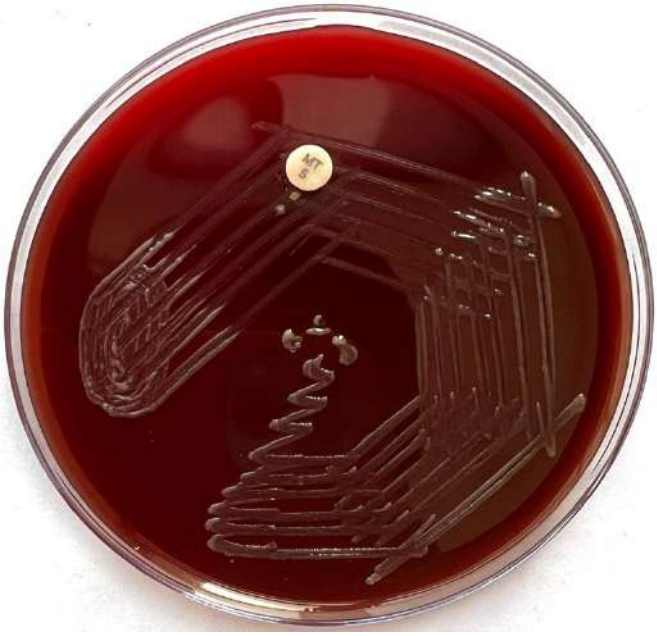


Image 6: Growth of *Prevotella spp* on Neomycin Blood Agar showing white, moist, circular, smooth colonies with 1-2 mm diameter.

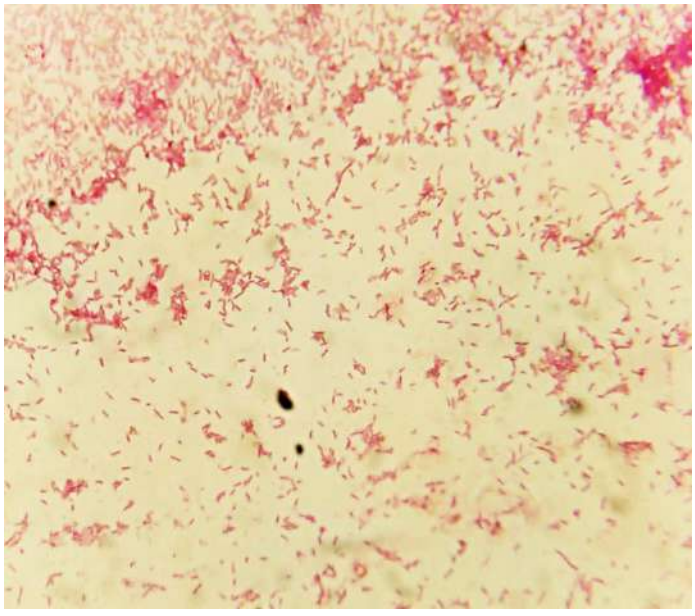


Image 7: Gram stain of *Prevotella spp* showing short gram negative non-sporulating bacilli.

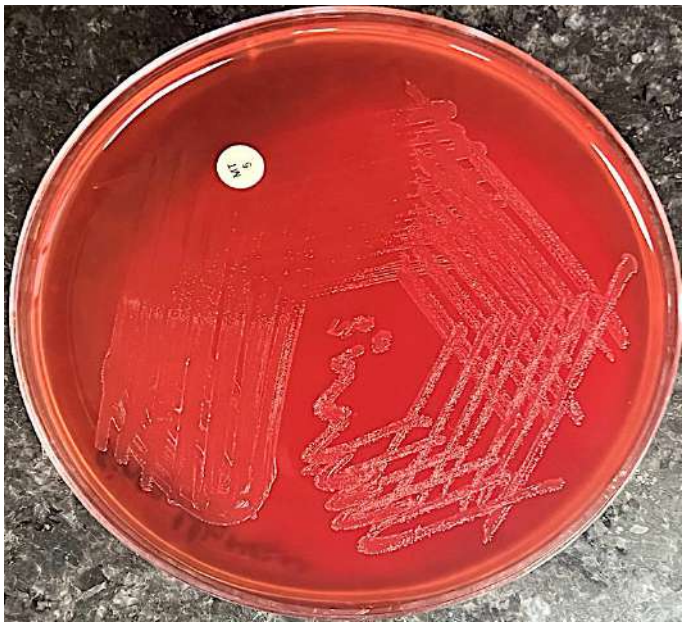


Image 8: Growth of *Parvimonas micra* on Brucella Blood Agar showing small, greyish-white, circular, smooth colonies with 0.5 - 1.5 mm diameter.

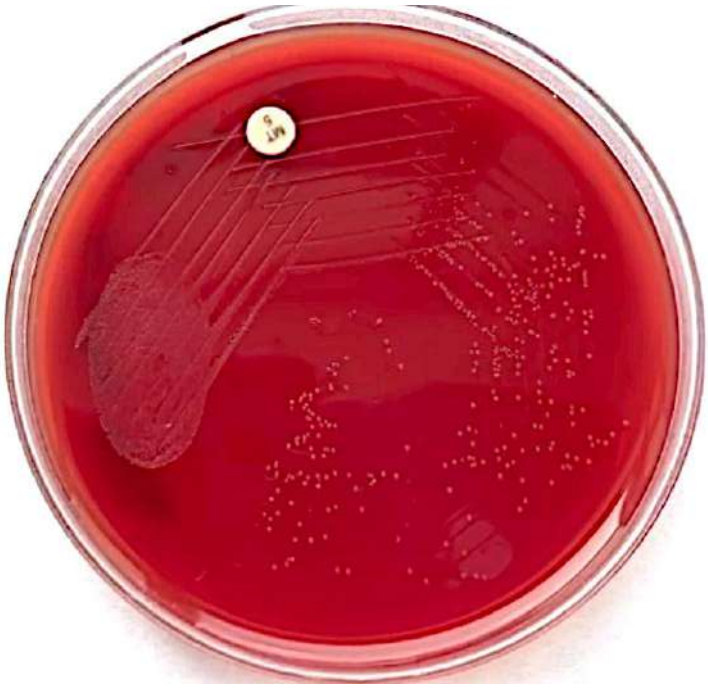


Image 9: Growth of *Parvimonas micra* on Neomycin Blood Agar showing pinpoint greyish white, circular, smooth colonies with 0.5 - 1 mm diameter.

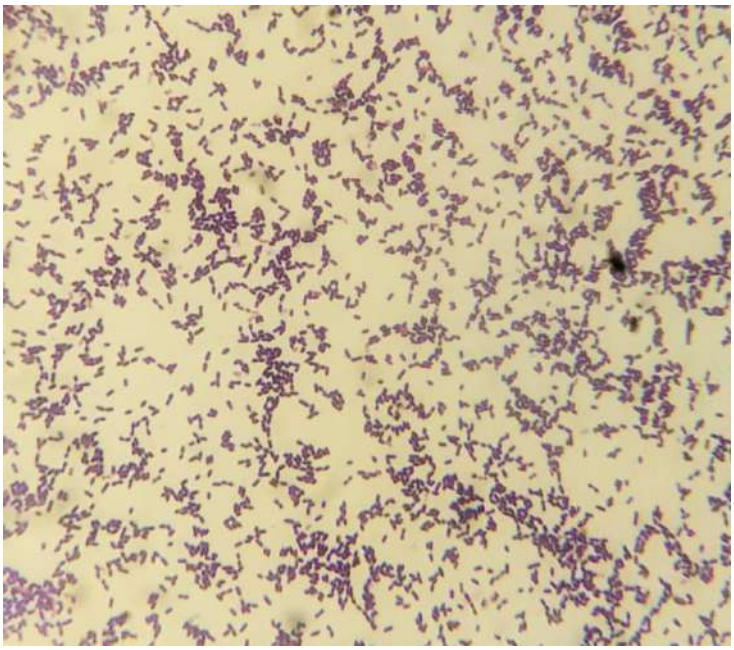


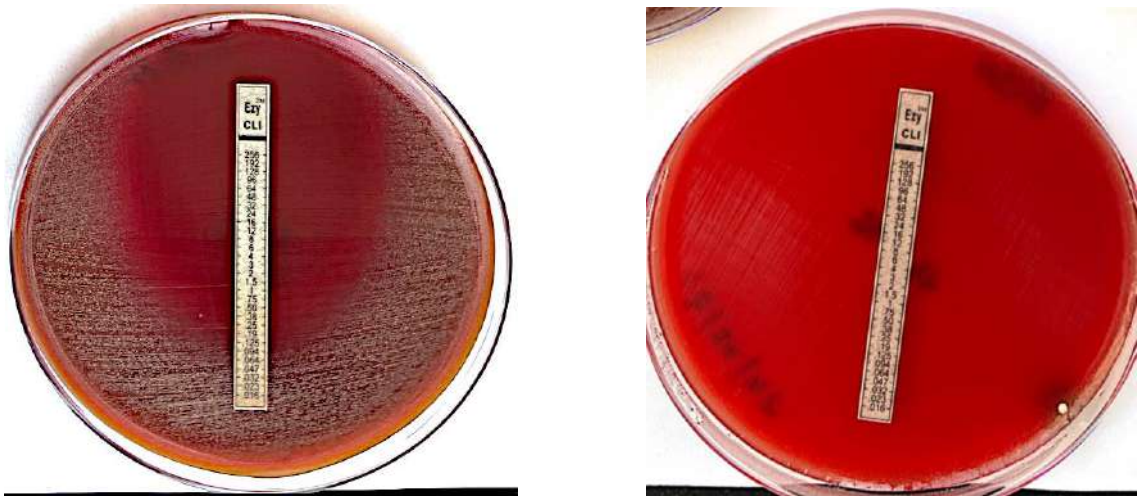
Image 10: Gram stain of *Parvimonas micra* showing gram positive cocci arranged in pairs and in short chains

Image 11: VITEK 2 Compact, (bioMerieux,USA)

A B

Image 12: Antimicrobial Susceptibility Testing of Metronidazole by E-strip

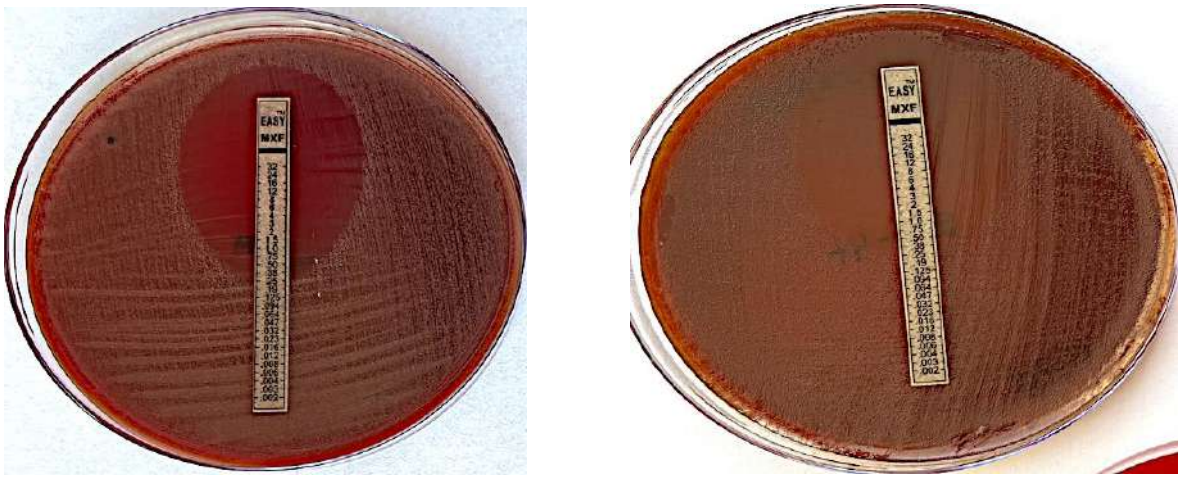
Image	MIC	Interpretation
A	≥ 256	R
B	0.25	S



A B

Image 13: Antimicrobial Susceptibility Testing of Clindamycin by E-strip

Image	MIC	Interpretation
A	0.125	S
B	≥ 256	R



A B

Image 14: **Antimicrobial Susceptibility Testing of Moxifloxacin by E-strip**

Image	MIC	Interpretation
A	0.25	S
B	≥ 256	R

A B

Image 15: Antimicrobial Susceptibility Testing of Tetracycline by E-strip

Image	MIC	Interpretation
A	0.25	S
B	≥ 256	R

A B

Image 16: Antimicrobial Susceptibility Testing of Ampicillin by E-strip

Image	MIC	Interpretation
A	0.19	S
B	≥ 256	R

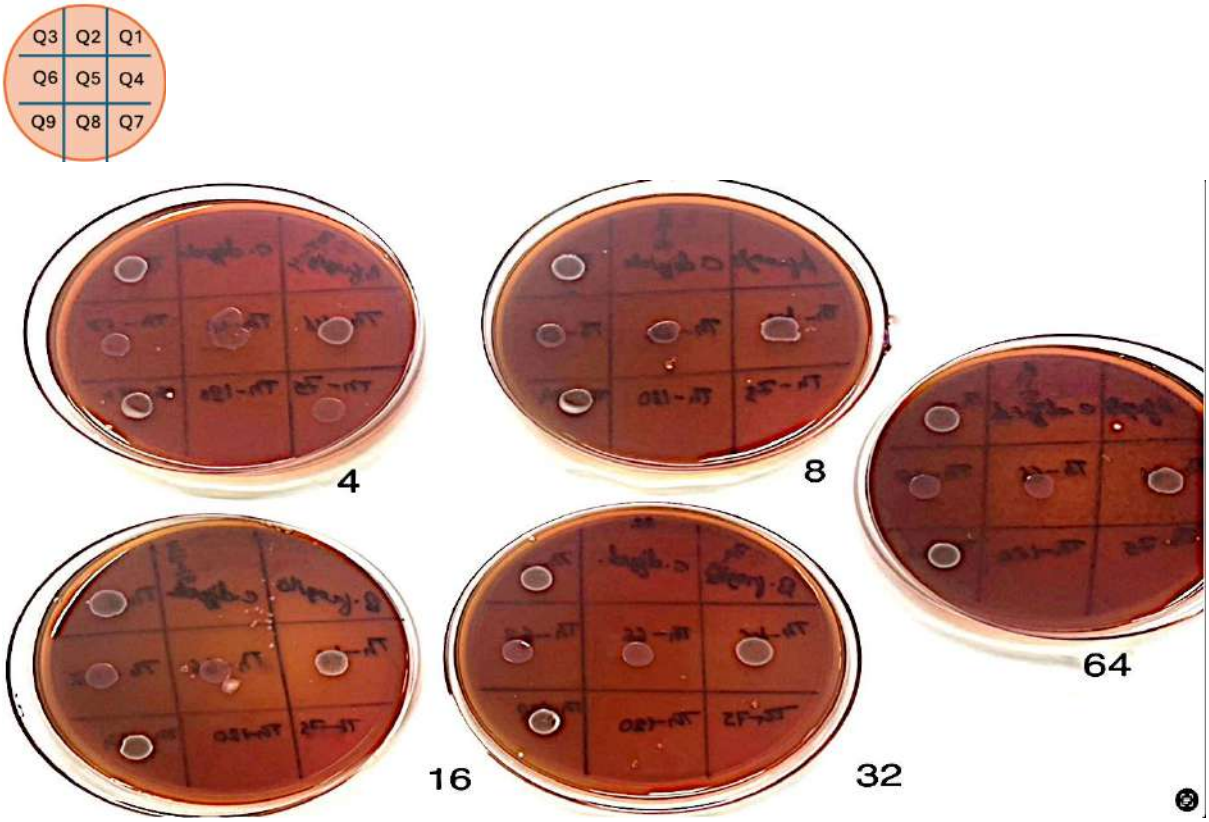


Image17: Antimicrobial Susceptibility Testing of Metronidazole by Agar Dilution Method

Q1- *Bacteroides fragilis ATCC 25285.* (MIC - ≤4) QC Pass ;

Q2- *Clostridium difficile ATCC 700057* (MIC - ≤4) QC Pass ;

Test Isolates : Q3- (MIC ≥64), R ; Q4-(MIC ≥64), R ; Q5-(MIC ≥64), R; Q6-(MIC≥64), R ; Q7-(MIC≤8), S ; Q8-(MIC ≤4), S ; Q9-(MIC ≥64), R.

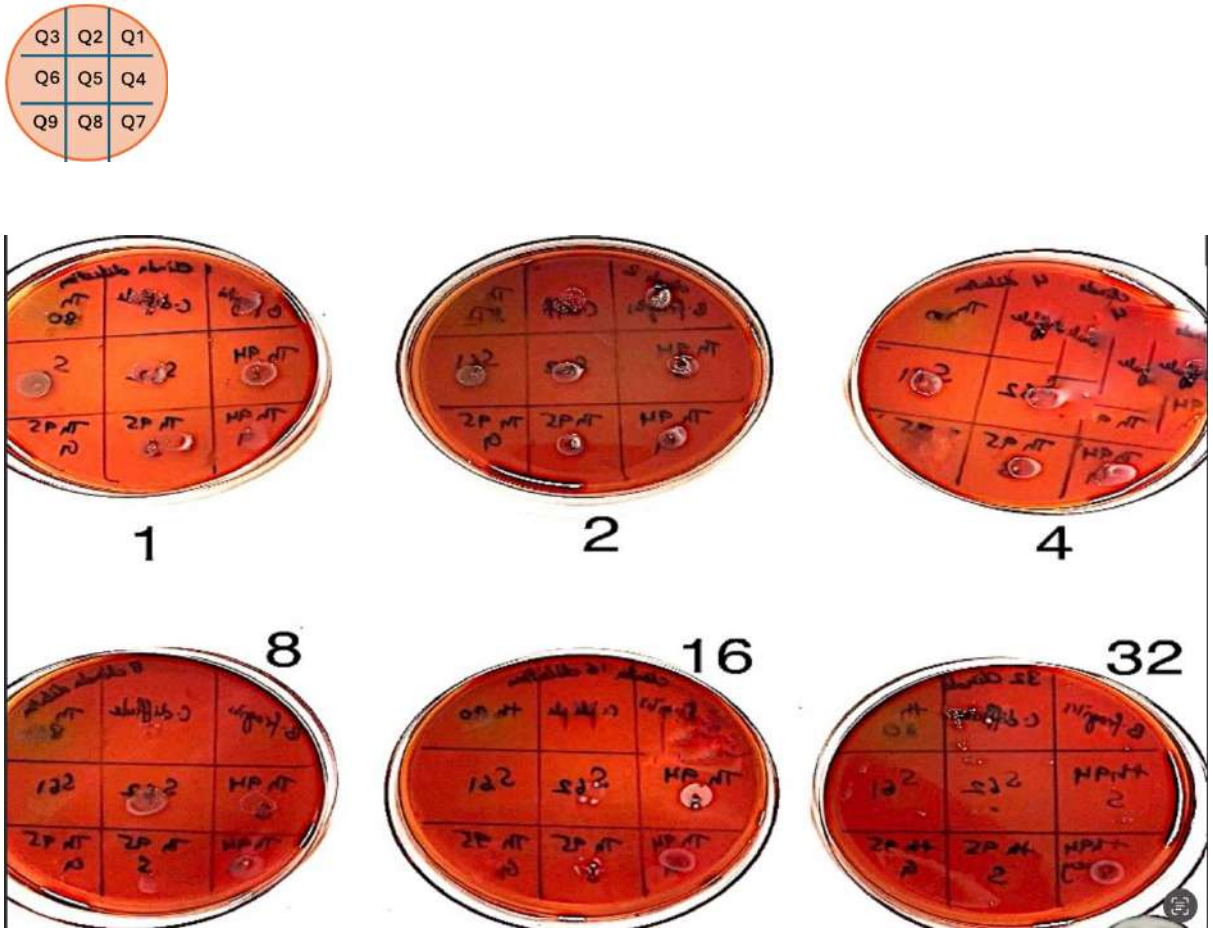


Image 18: Antimicrobial Susceptibility Testing of Clindamycin by Agar Dilution Method

Q1- *Bacteroides fragilis ATCC 25285.* (MIC - ≤2) QC Pass ;

Q2- *Clostridium difficile ATCC 700057* (MIC - ≤2) QC Pass ;

Test Isolates : Q3- (MIC ≤1), S ; Q4-(MIC ≥32), R ; Q5-(MIC ≥32), R; Q6-(MIC ≥8), R ;

Q7-(MIC ≥32), R ; Q8-(MIC ≥16), R ; Q9-(MIC ≤1), S.

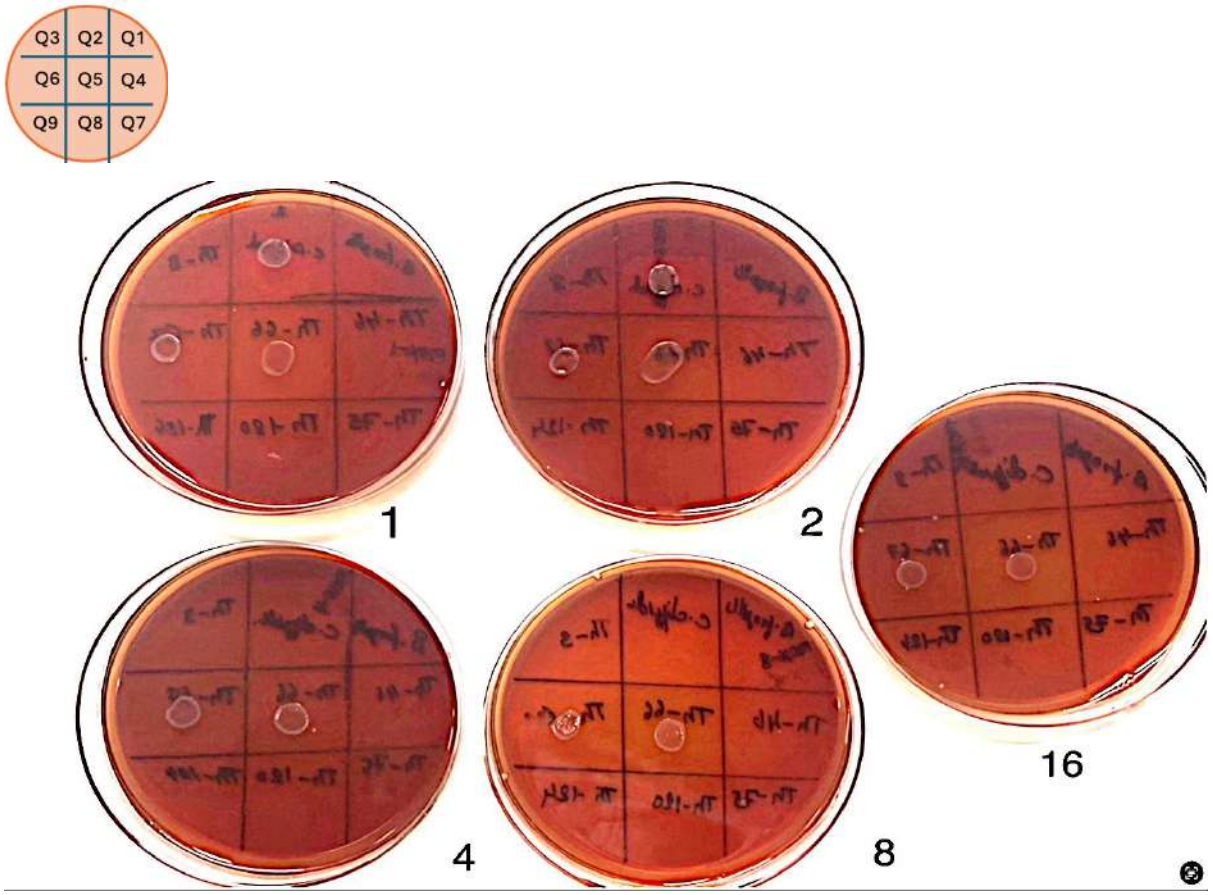


Image 19 : Antimicrobial Susceptibility Testing of Moxifloxacin by Agar Dilution Method

Q1- *Bacteroides fragilis ATCC 25285.* (MIC - ≤1) QC Pass ;

Q2- *Clostridium difficile ATCC 700057* (MIC - ≤2) QC Pass ;

Test Isolates : Q3- (MIC ≤1), S ; Q4-(MIC ≤1), S ; Q5-(MIC ≥16), R; Q6-(MIC ≥16), R ; Q7-(MIC ≤1), S ; Q8-(MIC ≤1), S ; Q9-(MIC ≤1), S.

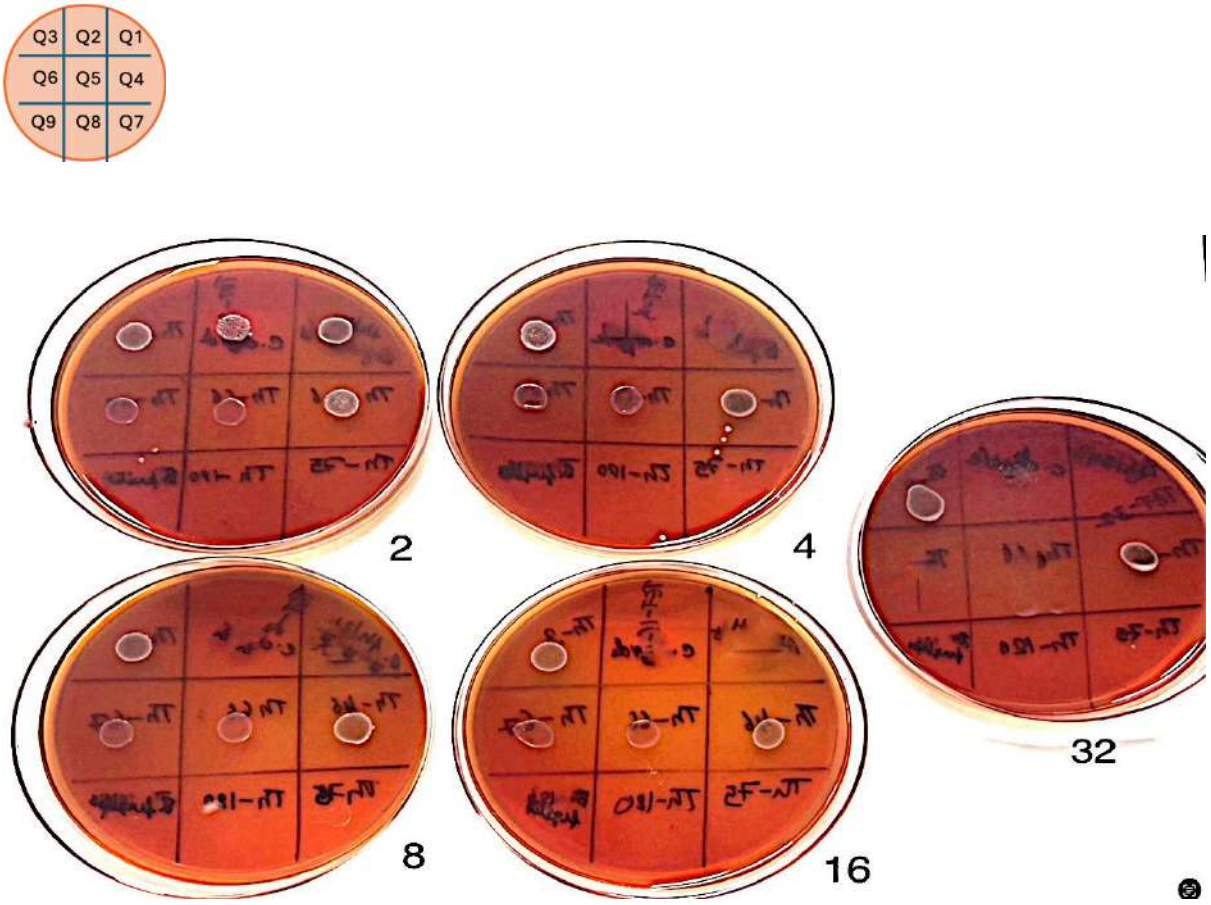


Image 20: Antimicrobial Susceptibility Testing of Tetracycline by Agar Dilution Method

Q1- *Bacteroides fragilis ATCC 25285.* (MIC - ≤2) QC Pass ;

Q2- *Clostridium difficile ATCC 700057* (MIC - ≤2) QC Pass ;

Test Isolates : Q3- (MIC ≥32), R ; Q4-(MIC ≥32), R ; Q5-(MIC ≥16), R; Q6-(MIC ≥16), R ; Q7-(MIC ≤1), S ; Q8-(MIC ≤1), S ; Q9-(MIC ≤1), S.

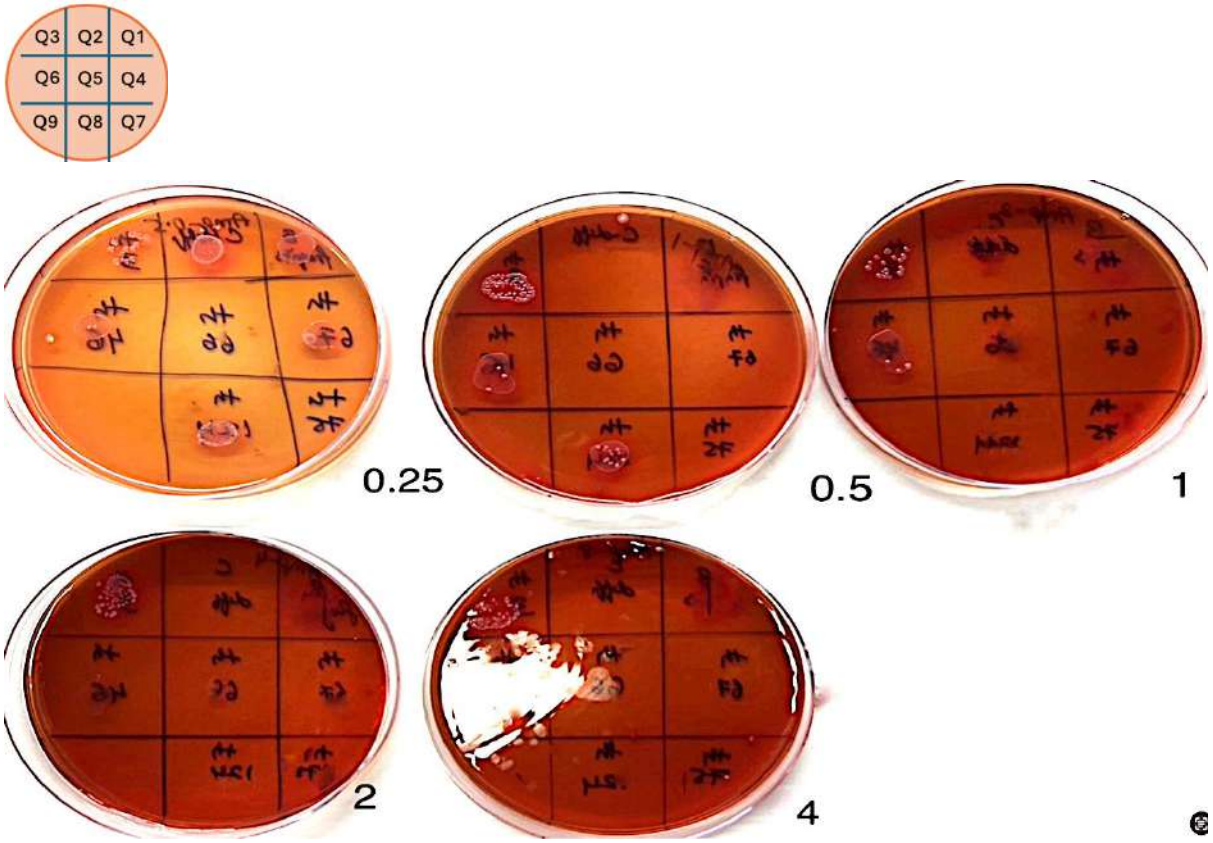


Image 21: Antimicrobial Susceptibility Testing of Ampicillin by Agar Dilution Method

Q1- *Bacteroides fragilis ATCC 25285.* (MIC - ≤0.5) QC Pass ;

Q2- *Clostridium difficile ATCC 700057* (MIC - ≤0.5) QC Pass ;

Test Isolates : Q3- (MIC ≥4), R ; Q4-(MIC ≤0.5), S ; Q5-(MIC ≤0.25), S;

Q6-(MIC ≥1), R ; Q7-(MIC- ≤0.25), S ; Q8-(MIC ≤0.5), S.

Table 8: Distribution of anaerobes among study participants (n=200)

	Obligate anaerobes	Facultative anaerobes	No Growth	Total
Dental caries (n=100)	14	64	22	100
Periodontitis (n=100)	16	62	22	100
Total	**30 (15%)**	**126 (63%)**	**44 (22%)**	**200**

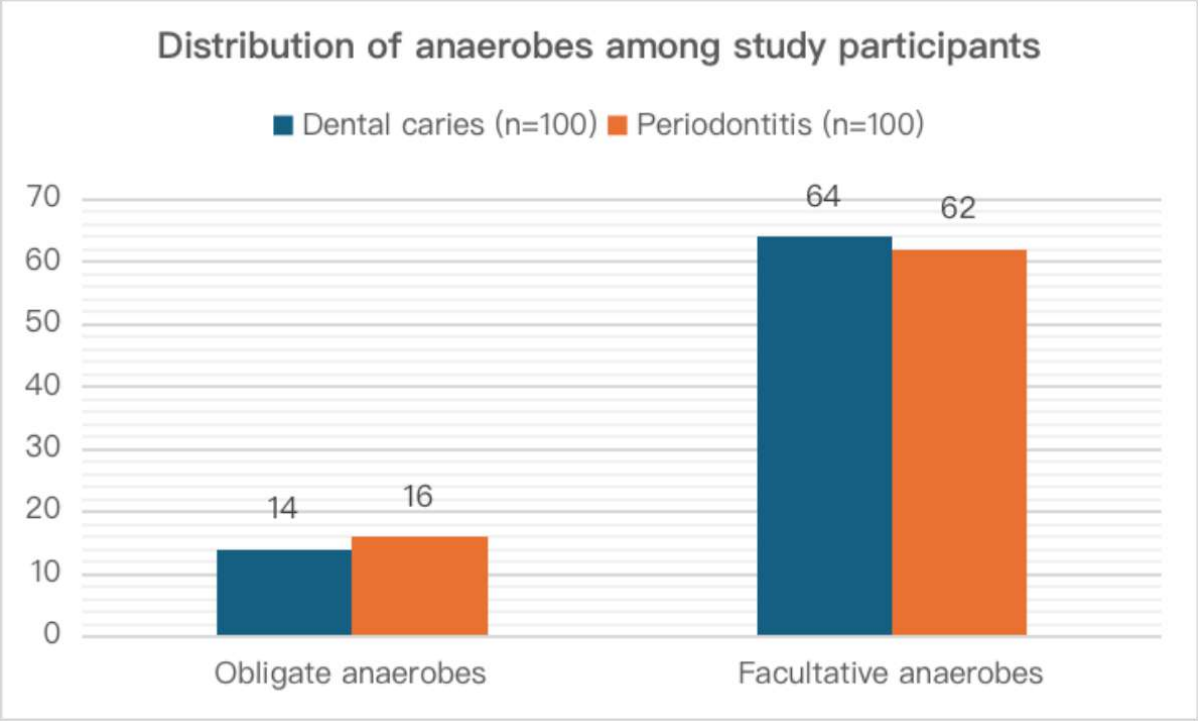


Figure 1: Distribution of anaerobes among study participants (n=200)

Table 9: Age wise distribution of study participants (n=200)

Age group	Cases of orodental infections with obligate anaerobes n(%)	Cases of orodental infections without obligate anaerobes n(%)	Total n(%)
5-18 years	0	16 (8%)	**16 (8%)**
19-36 years	15 (7.5%)	101 (50.5%)	**116 (58%)**
37-54 years	11 (5.5%)	33 (16.5%)	**44 (22%)**
55-72 years	3 (1.5%)	20 (10%)	**23 (11.5%)**
> 72 years	1 (0.5%)	0	**1 (0.5%)**
Mean age in years	38.0±16.09	33.04±14.64	-
Total	**30 (15%)**	**170 (85%)**	**200 (100%)**

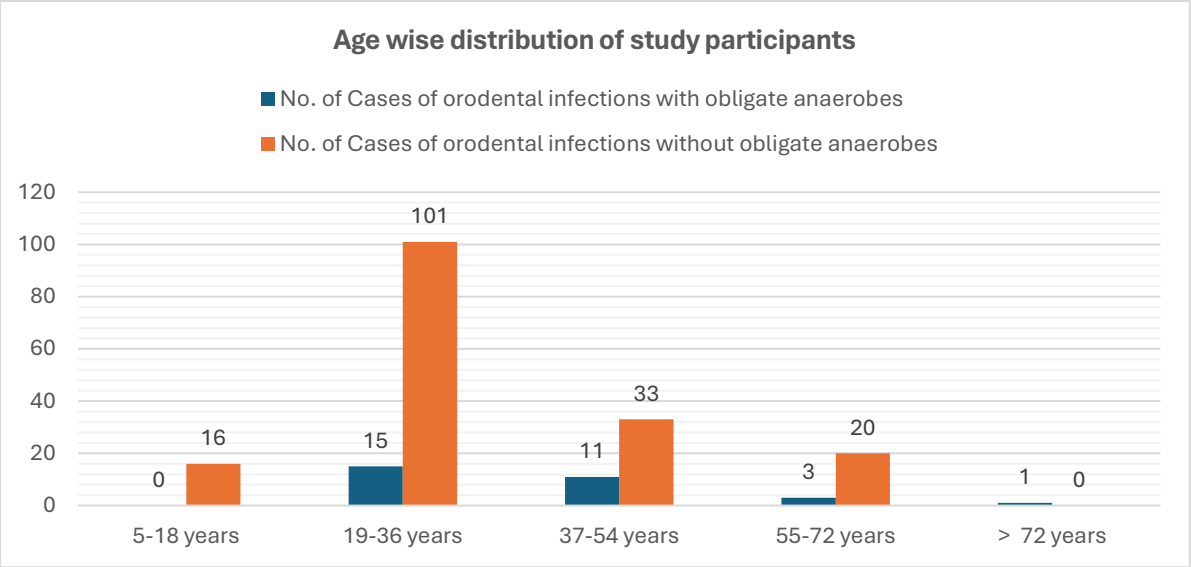


Figure 2: Age wise distribution of study participants (n=200)

Table 10: Gender wise distribution of study participants (n=200)

Gender	Cases of orodental infections with obligate anaerobes n(%)	Cases of orodental infections without obligate anaerobes n(%)	Total n(%)	p value
Male	24 (12%)	84 (42%)	**108 (54%)**	**< 0.01**
Female	6 (3%)	86 (43%)	**92 (46%)**	
Total	**30 (15%)**	**170 (85%)**	**200**	

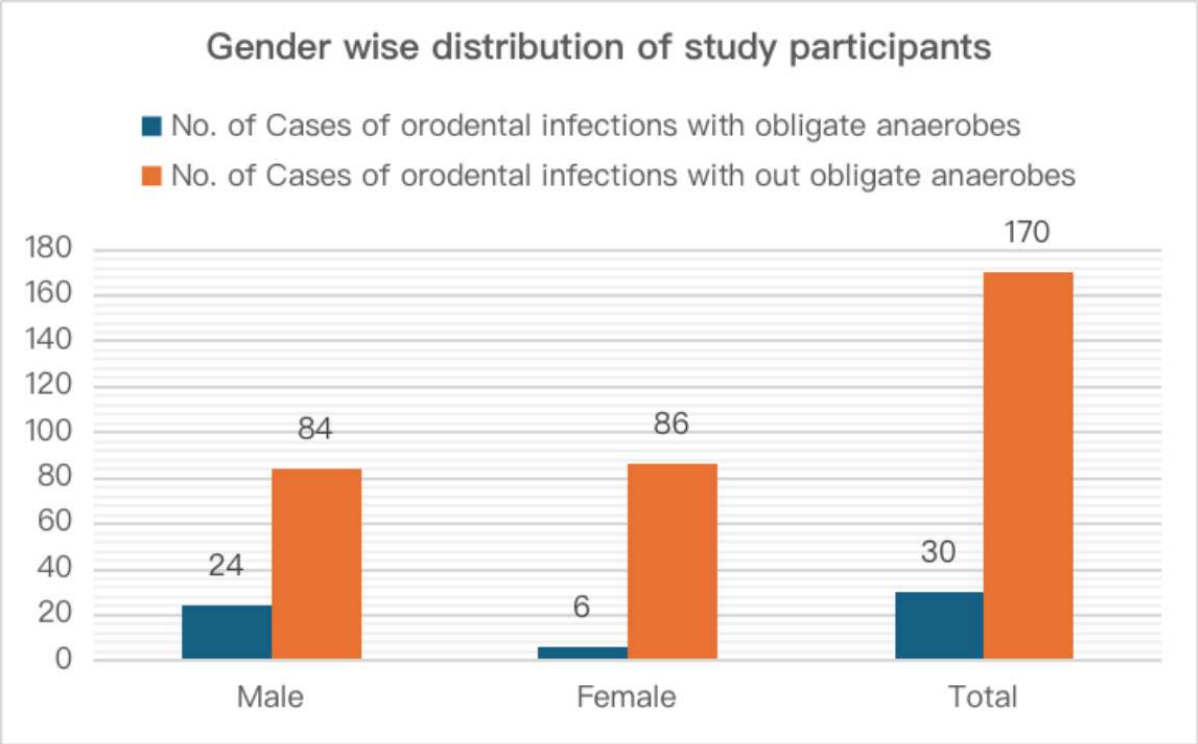


Figure 3: Gender wise distribution of study participants (n=200)

Table 11: Chief complaints among study participants (n=200)

Chief complaints	Cases of orodental infections with obligate anaerobes (30) n(%)	Cases of orodental infections without obligate anaerobes (170) n(%)	Total (n=200) n(%)	P value
Tooth sensitivity to hot & Cold temperature	30 (100%)	151(88.8%)	**181(90.5%)**	0.45
Tooth ache	28 (93.3%)	151(88.8%)	**179 (89.5%)**	**0.05**
Recurrent episodes of infection	14 (46.6%)	39 (22.9%)	**53 (26.5%)**	**0.01**
Pain/discomfort while chewing	12 (40%)	86 (50.6%)	**98 (49%)**	0.28
Difficulty in swallowing	1(3.3%)	8(4.7%)	**9 (4.5%)**	0.73
Swelling of face/neck/cheek	0(0.0%)	2(1.2%)	**2 (1%)**	0.55

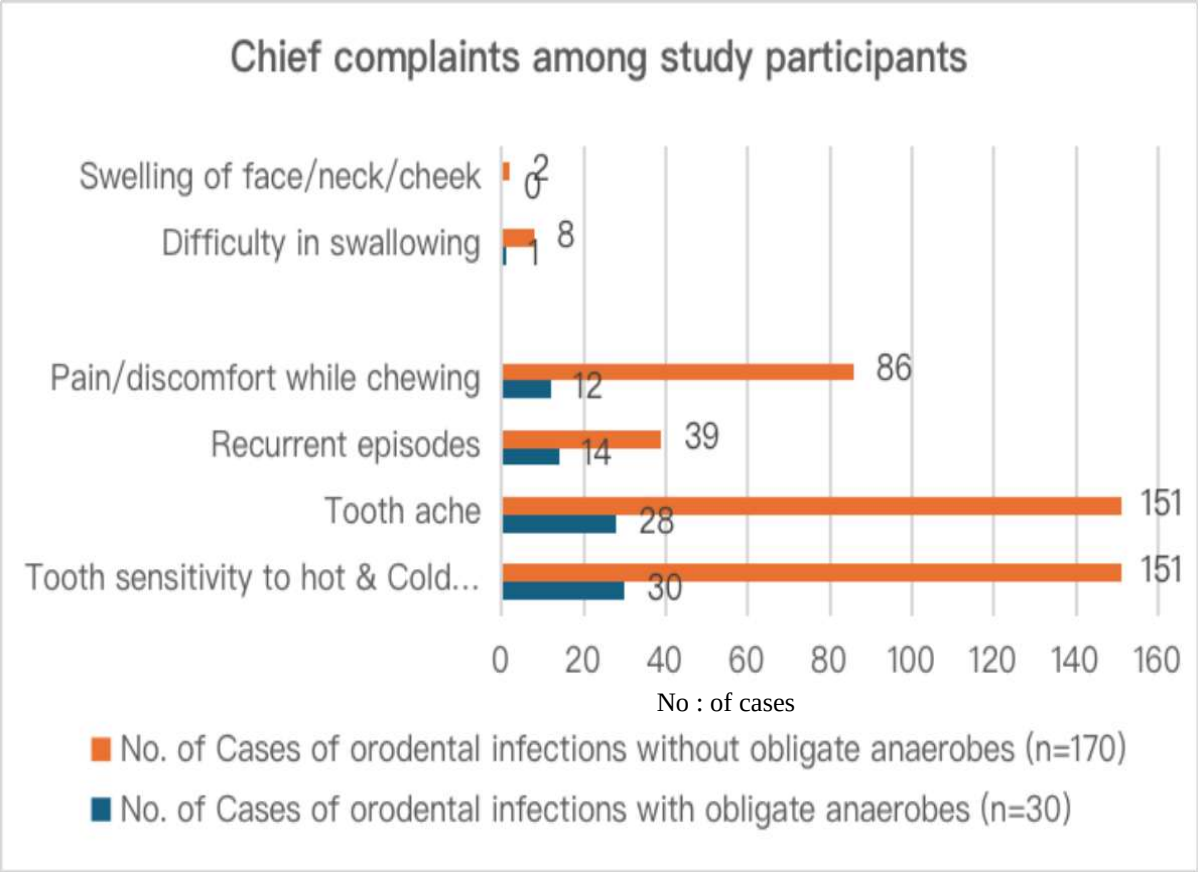


Figure 4: Chief complaints among study participants (n=200)

Table 12 : Risk factors among study participants (n=200)

Risk factors	Cases of orodental infections with obligate anaerobes (30) n(%)	Cases of orodental infections without obligate anaerobes (170) n(%)	Total (n=200) n(%)	P value
Diabetes Mellitus	8 (26.7%)	21 (12.4%)	**29 (14.5%)**	**0.04**
Invasive oral procedures	1 (3.3%)	1 (0.5%)	**2 (1%)**	0.16
Malignancies	0(0.0%)	1(0.6%)	**1(0.5%)**	1.0

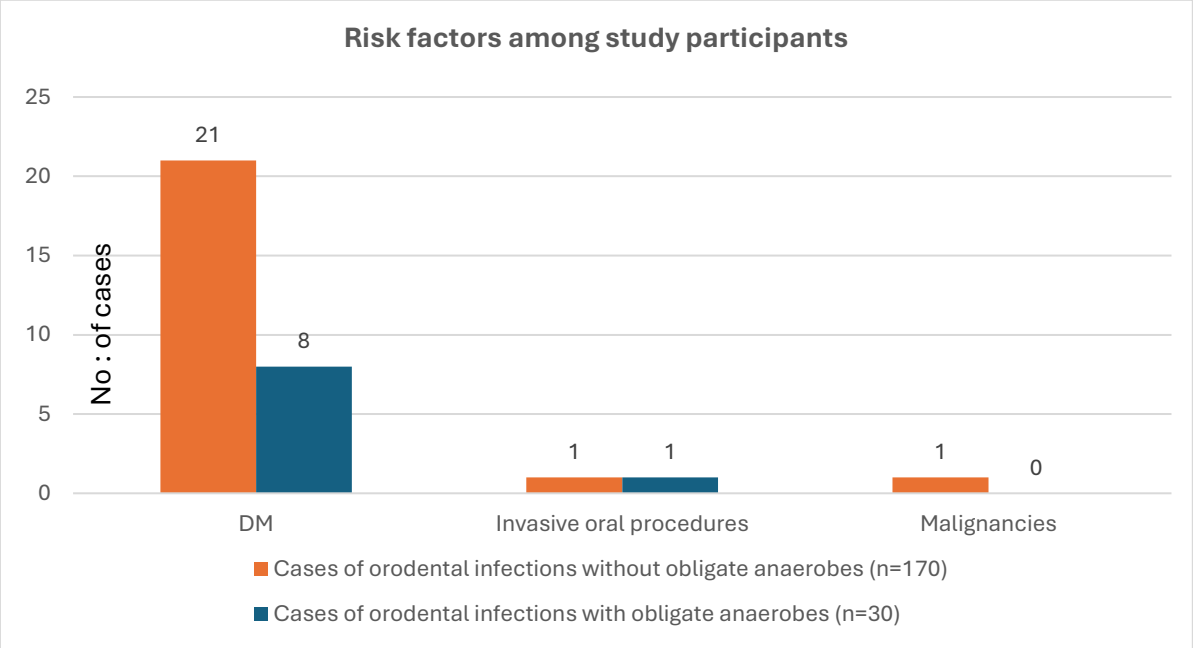


Figure 5: Risk factors among study participants (n=200)

Table 13: Distribution of obligate anaerobes among study participants (n=30)

Type of obligate anaerobe	Dental caries (n=14)	Periodontitis (n=16)	Total (n=30) n (%)
Gram negative obligate anaerobes			
Prevotella oris	4	4	8 (26%)
Prevotella buccae	2	2	4 (13.3%)
Prevotella disiens	3	0	3 (10%)
Prevotella oralis	0	2	2 (6.6%)
Fusobacterium nucleatum	0	1	1 (3.3%)
Bacterioides ovatus	1	0	1 (3.3%)
B fragilis	0	1	1 (3.3%)
Total	**10**	**10**	**20 (66.6%)**
Gram positive obligate anaerobes			
Parvimonas micra	1	2	3 (10%)
Peptoniphilus assacharolyticus	1	2	3 (10%)
Clostridium clostridioforme	1	1	2 (6.6%)
Peptostreptococcus anaerobius	1	0	1 (3.3%)
Anaerococcus prevotii	0	1	1 (3.3%)
Total	**4**	**6**	**10 (33.33%)**
Grand total	**14**	**16**	**30**

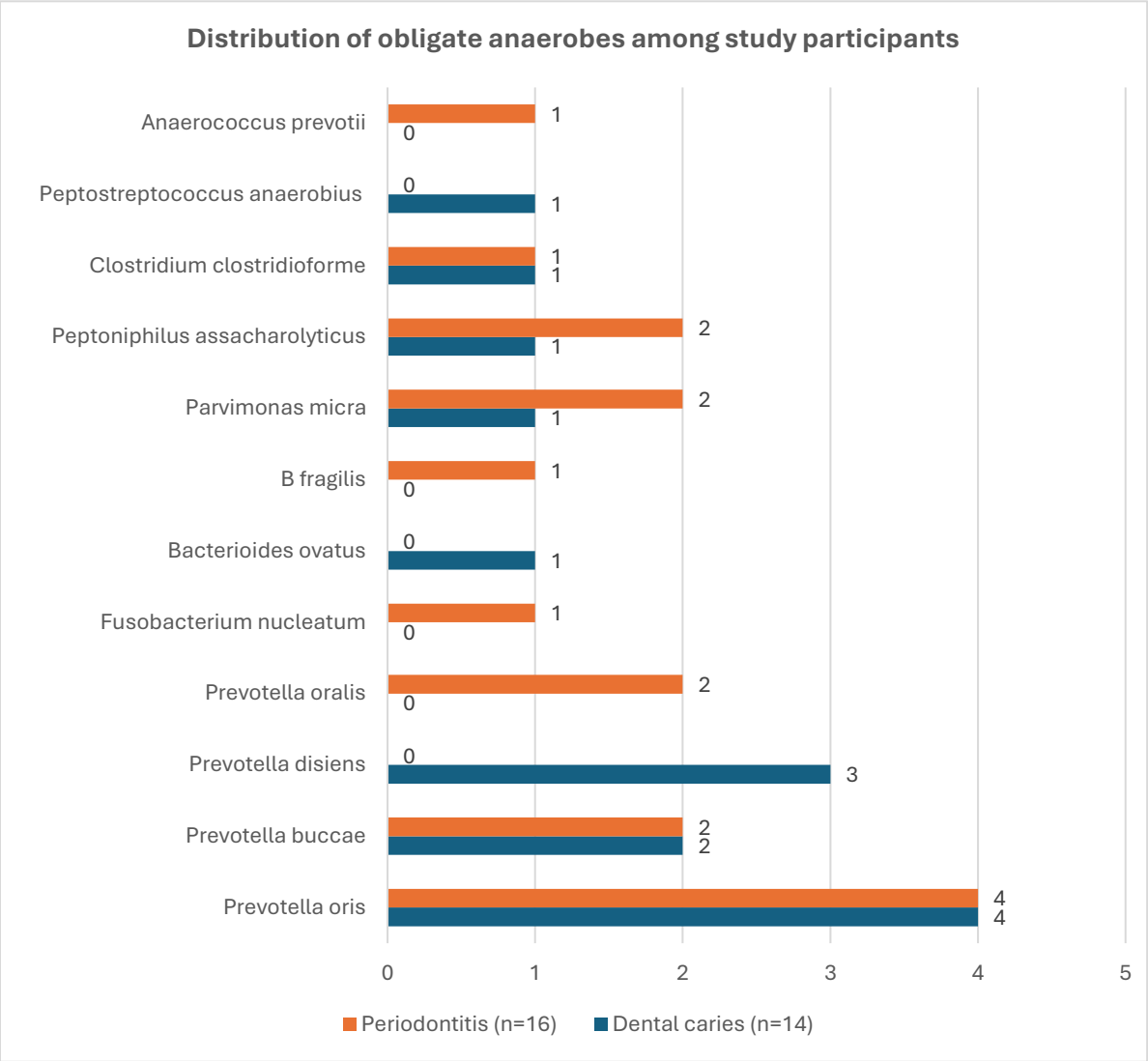


Figure 6: Distribution of obligate anaerobes among study participants (n=30)

Table 14: Antimicrobial susceptibility profile of obligate anaerobes by E-strip method(n=30)

Obligate anaerobic isolates	Antimicrobial susceptibility testing by E-strip method (n=30)									
	Moxifloxacin		Clindamycin		Metronidazole		Tetracycline		Ampicillin	
Gram negative anaerobes	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Prevotella oris (n=8)	7	1	8	0	4	4	5	3	3	5
Prevotella buccae (n=4)	4	0	3	1	4	0	3	1	2	2
Prevotella disiens (n=3)	3	0	3	0	2	1	3	0	1	2
Prevotella oralis (n=2)	2	0	1	1	1	1	1	1	1	1
Bacteroides ovatus (n=1)	0	1	1	0	1	0	0	1	0	1
Fusobacterium nucleatum (n=1)	1	0	1	0	1	0	1	0	1	0
B fragilis (n=1)	1	0	1	0	1	0	1	0	0	1
Total (n=20)	**18**	**2**	**18**	**2**	**14**	**6**	**14**	**6**	**8**	**12**
Gram positive anaerobes										
Parvimonas micra (n=3)	3	0	2	1	1	2	1	2	3	0
Peptoniphilus assacharolyticus(n=3)	2	1	2	1	0	3	0	3	2	1
Clostridium clostridioforme (n=2)	2	0	1	1	2	0	1	1	0	2
Peptostreptococcus anaerobius(n=1)	1	0	1	0	0	1	1	0	1	0
Anaerococcus prevotii (n=1)	1	0	0	1	1	0	0	1	1	0
Total (n=10)	**9**	**1**	**6**	**4**	**4**	**6**	**3**	**7**	**7**	**3**
Grand Total (n=30)	**27**	**3**	**24**	**6**	**18**	**12**	**17**	**13**	**15**	**15**
Percentage %	**90%**	**10%**	**80%**	**20%**	**60%**	**40%**	**56.6%**	**43.3%**	**50%**	**50%**

Note: Intermediate sensitivity was not observed for any of the isolates.

Table 15: Antimicrobial susceptibility profile of obligate anaerobes by Agar dilution method(n=15)

Obligate anaerobic isolates	Antimicrobial susceptibility testing by Agar dilution method (n=15)									
	Moxifloxacin		Clindamycin		Metronidazole		Tetracycline		Ampicillin	
Gram negative anaerobes	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Prevotella oris (n=8)	6	2	6	2	4	4	6	2	4	4
Prevotella disiens (n=2)	2	0	2	0	1	1	2	0	0	2
Bacteroides fragilis (n=1)	1	0	1	0	1	0	1	0	0	1
Total (n=11)	**9**	**2**	**9**	**2**	**6**	**5**	**9**	**2**	**4**	**7**
Gram positive anaerobes										
Peptoniphilus assacharolyticus(n=2)	2	0	2	0	0	2	0	2	2	0
Parvimonas micra (n=1)	1	0	0	1	1	0	0	1	1	0
Clostridium clostridioforme(n=1)	1	0	0	1	1	0	1	0	0	1
Total (n=4)	**4**	**0**	**2**	**2**	**2**	**2**	**1**	**3**	**3**	**1**
Grand Total (n=15)	**13**	**2**	**11**	**4**	**8**	**7**	**10**	**5**	**7**	**8**
Percentage %	**86.6%**	**13.3%**	**73.3%**	**26.6%**	**53.3%**	**46.6%**	**66.6%**	**33.3%**	**46.6%**	**53.3%**

Note: Intermediate sensitivity was not observed for any of the isolates.

Table 16: Comparison of AST results by E-strip method and Agar dilution method (n=15)

Antimicrobial agents	Results of AST by E-strip method		Results of AST by Agar dilution method		% Concordance of E-strip results with agar dilution results	
	S	R	S	R	S	R
Moxifloxacin	14	1	13	2	92.8%	50%
Clindamycin	11	4	11	4	100%	100%
Metronidazole	8	7	8	7	100%	100%
Tetracycline	10	5	10	5	100%	100%
Ampicillin	7	8	7	8	100%	100%

Note: Intermediate sensitivity was not observed for any of the isolates.

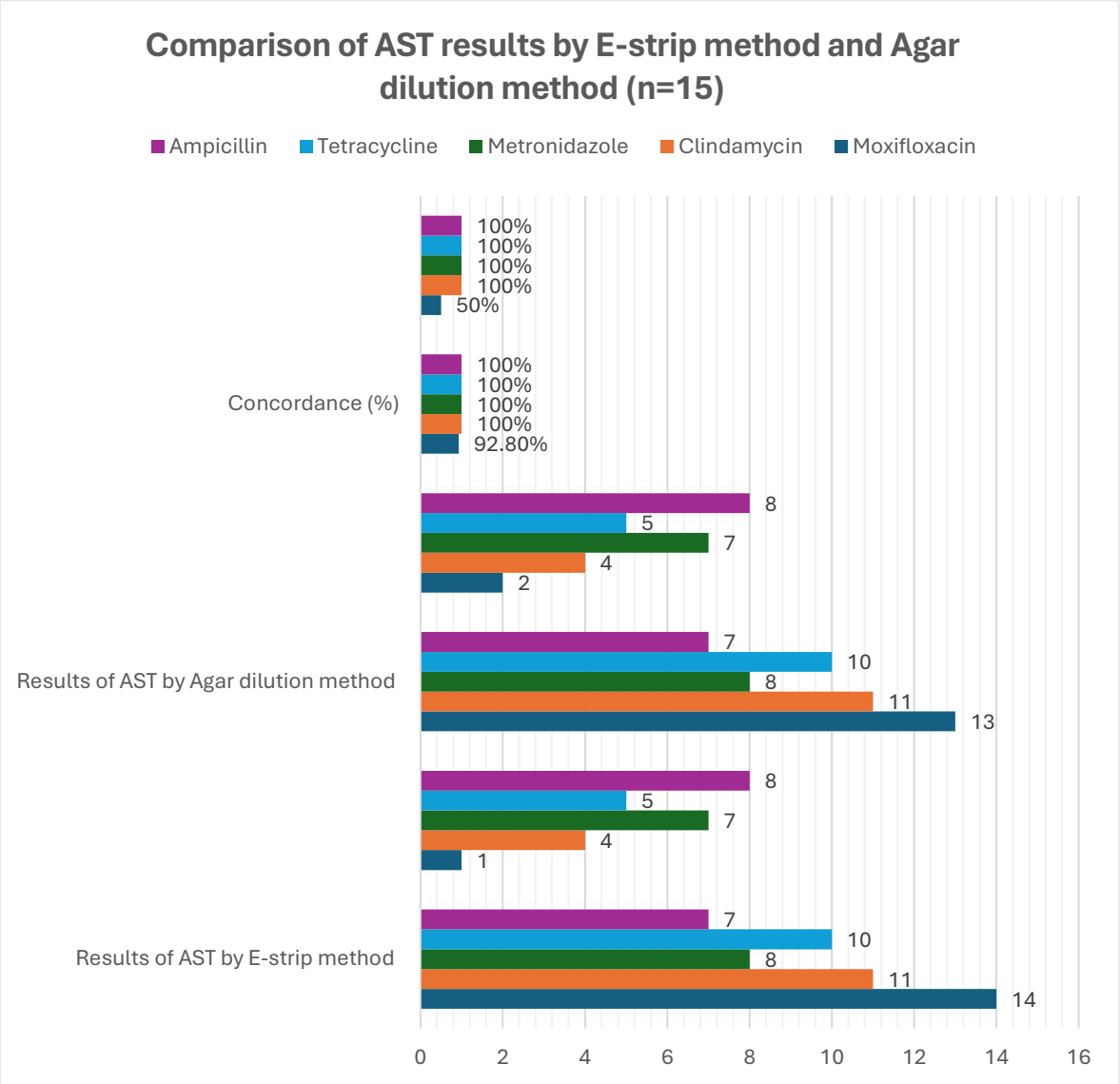


Figure 7: Comparison of AST results by E-strip method and Agar dilution method (n=1

Table 17: Comparative analysis of AST results by E-strip method with Agar dilution method as gold standard for Moxifloxacin (n=15)

		Agar Dilution Method	
E-Strip Method		S	R
	S	13	1
	R	0	1

Sensitivity & NPV**:** 100%

Specificity: 50%

Accuracy: 93.33%

Table 18: Comparative analysis of AST results by E-strip method with Agar dilution method as gold standard for Clindamycin (n=15)

		Agar Dilution Method	
E-Strip Method		S	R
	S	11	0
	R	0	4

Sensitivity, Specificity, PPV, NPV, & Accuracy: 100%

Table 19: Comparative analysis of AST results by E-strip method with Agar dilution method as gold standard for Metronidazole (n=15)

		Agar Dilution Method	
E-Strip Method		S	R
	S	8	0
	R	0	7

Sensitivity, Specificity, PPV, NPV, & Accuracy: 100%

Table 20: Comparative analysis of AST results by E-strip method with Agar dilution method as gold standard for Tetracycline (n=15)

		Agar Dilution Method	
E-Strip Method		S	R
	S	10	0
	R	0	5

Sensitivity, Specificity, PPV, NPV, & Accuracy: 100%

Table 21: Comparative analysis of AST results by E-strip method with Agar dilution method as gold standard for Ampicillin (n=15)

		Agar Dilution Method	
E-Strip Method		S	R
	S	7	0
	R	0	8

Sensitivity, Specificity, PPV, NPV, & Accuracy: 100%

DISCUSSION

Discussion:

The results of the present study highlights the importance of obligate anaerobes among patients with orodental infection with significant emergence of antimicrobial resistance for commonly prescribed antimicrobial agents. 200 recruited participants overall , facultative anaerobes were the most prevalent, identified in 126 individuals (63%) [Table 8;Figure 1] , while obligate anaerobes were detected in 30 participants (15%), In the dental caries group, 14 (14%) had obligate anaerobes, 64 (64%) had facultative anaerobes. In the periodontitis group, obligate anaerobes are 16 (16%) and facultative anaerobes are 62 (62%). The global prevalence rates typically ranging between 15% to 40%.[163,164,165] A study by Bansal S et al noted that the presence of obligate anaerobic bacteria was prevalent in both dental caries and periodontitis.[164] Study conducted by brook I et al reported a prevalence of 12-17% for obligate anaerobes in orodental infections.[163] Siqueira JF et al found a prevalence rate of 25 – 40 % for obligate anaerobes causing orodental infections. [165,166] A study conducted by Niazi SA et al found that prevalence rate of 20% for obligate anaerobes in patients with orodental infections.[167] Baquero F et al and Jacinto RC et al reported a prevalence rate of 33% for obligate anaerobes in orodental infections. [168] The observations of the present study shows that facultative anaerobes play a crucial role in dental caries and periodontitis indicates infection. Facultative anaerobes, unlike obligate anaerobes, can survive and proliferate in both oxygen-rich and oxygen-deprived environments, which makes them highly adaptable to the fluctuating conditions in the oral cavity. This adaptability allows them to thrive in dental plaque, gingival crevices, and periodontal pockets, which experience varying oxygen levels depending on oral hygiene, inflammation, and tissue destruction. .[163,164] Rams TE et al reported that obligate anaerobes were isolated in 15-20% of patients with periodontitis, with Peptostreptococcus species being common. [169] Brazier JS et al reported in his study that obligate anaerobes were isolated in 16-24% of orodental infection samples. [170] In a study conducted by Al-Haroni M et al reported a prevalence rate of 22% for obligate anaerobes in patients with periodontitis. [171] In general, recent studies show prevalence rates of obligate anaerobes ranging from 15% to 30% in different populations.[172] Study by Kumar et al**.** found that obligate anaerobes were isolated in 18.5% of cases of periodontitis and dental abscesses, with a slight increase in comparison to earlier years due to improved anaerobic culturing techniques. [172] A 2021 study by Almeida et al**.** in Brazil reported a prevalence rate of 22% in orodental infections, primarily in periodontal disease patients, marking an increase from earlier data due to better recognition of polymicrobial infections. [173] Study from

China, Chen et al**.** found a 15% prevalence of obligate anaerobes in endodontic infections, emphasizing the association between these organisms and chronic dental conditions. [174]

In the present study majority of the infections were reported in the age group of 19-36 years, accounting for 58% of all infections with maximum isolation rate for obligate anaerobes(7.5%), followed by 37-54 years and 55-72 years age groups.[Table 9;Figure2] showing lower infection rates (22% and 11.5%, respectively). However, only 8% infections were observed in paediatric age group. The mean age of participants with and without obligate anaerobes indicate that the mean age for those with positive obligate anaerobe organisms was 38.0 years (±16.09), compared to 33.04 years (±14.64) for those without obligate anaerobes. In a study conducted by Khan et al. (2018) examined orodental infections and found a higher prevalence in the younger age group, consistent with the present study findings, where this age group also showed significant representation (41.0%).[175] Singh et al. reported that patients aged 21-40 years accounted for 60% of obligate anaerobic infections, emphasizing the relevance of young adults in both studies.[176] Study by Gupta et al. showed similar trends, where the majority of anaerobic infections occurred in the 20-30 years range, with 45% prevalence, aligning closely with the present study findings. [177] Sharma et al. noted a significant prevalence (35%) in the 21-30 years group, reinforcing the present study results indicating this demographic as the most affected .[178] In a study conducted by Verma et al. indicated that the majority of obligate anaerobic orodental infections occurred in individuals aged 21-35 years, paralleling the present study's peak prevalence in the 21-30 years age group .[179] In a study by Patel et al. 30% of cases were found in the 30-40 years age group, indicating a trend where young adults remain at risk for anaerobic infections.[180] Jain et al. found that only 5% of cases were in the >70 years age group, consistent with the present study's findings that also showed low prevalence in older adults. [181] Suresh et al. reported a similar trend, with 40% of their subjects aged between 21-30 years, highlighting the vulnerability of this demographic to anaerobic infections. [182] Over the past five years, the prevalence of obligate anaerobes in orodental infections has shown significant trends in age distribution. As the years progress, the trends in the prevalence of obligate anaerobes in orodental infections are evolving significantly. [165] This trend indicates changing patterns in susceptibility, possibly due to evolving risk factors like smoking, dietary habits etc associated with orodental health. The results in the present study, showing the highest prevalence of orodental infections with obligate anaerobes

in the 19-36 years age group, can be attributed to several lifestyle and physiological factors.[165] This age group is often exposed to significant risk factors such as smoking, poor oral hygiene, a diet high in refined sugars, and inconsistent dental care practices. These factors contribute to the creation of an anaerobic environment in the oral cavity, promoting the growth of obligate anaerobes like *Porphyromonas gingivalis* and *Fusobacterium nucleatum.*[165]

In the present study, significant difference in the orodental infections was observed among male to female (p=<0.01).[Table 10; Figure 3]Infection with obligate anaerobes was found in 12% of male patients and only 3% of female patients with orodental infections. This trend aligns with findings from various studies conducted so far. Dore et al. found that 78% of patients with anaerobic orodental infections were male, suggesting a similar male-to-female ratio in their cohort.[183] Additionally, Sharma et al. noted that 76% of their obligate anaerobe-positive patients were male, highlighting a consistent gender disparity.[184] Study conducted by Prakash et al. showed, males constituted 82% of patients with anaerobic orodental infections, which is comparable to the findings of the current study.[185] A research by Jain et al. documented a male predominance of 79%, reinforcing the notion that males are more frequently affected by these infections.[186] Ranjan et al. observed that 74% of the obligate anaerobic orodental infection cases were male, aligning closely with the present study's results.[187] Other studies, such as those conducted by Gupta et al. and Singh et al. found male predominance at rates of 75% and 77%, respectively, corroborating the trends noted in the current research. [188,189] A recent meta-analysis by Ranjit and Kumar (2024) indicated that the overall male prevalence in anaerobic orodental infections ranges from 70% to 82%, emphasizing a consistent trend in gender distribution across different studies.[190,191] The observed male preponderance in orodental infections caused by obligate anaerobes can be attributed to several interrelated factors.[191] Anatomical and physiological differences, particularly hormonal variations such as testosterone levels, may influence immune response and susceptibility to infections.[191] Behavioral factors also play a significant role. It has been observed that males often engage in riskier health behaviors, including poor oral hygiene, higher rates of tobacco and alcohol use, and less frequent dental visits, which can lead to an increased incidence of dental caries and periodontal disease.[191]

In the present study the clinical symptoms were compared among the patients with orodental infections with obligate anaerobes and the patients without obligate anaerobes. Significant difference was noted for the tooth ache (p=0.05)[Table 11;Figure 4] and recurrent episodes of infection(p=0.01). Among the participants, tooth sensitivity to hot and cold temperatures was the most common complaint observed. Additionally, toothache was reported in 93.3% of cases with obligate anaerobes compared to 88.8% without (P = 0.05), indicating a near-significant association that highlights the potential severity of these infections between the two groups. Overall, the findings suggest that orodental infections with obligate anaerobes are more likely to be associated with recurrent episodes and, to a lesser extent, toothache, indicating a potentially more aggressive or persistent infection pattern. These findings align with a study by Kaur et al, which found that 95% of patients with anaerobic infections experienced significant pain, often exacerbated by thermal stimuli.[192] Similarly, Muliya et al. found that 92% of patients complained of toothache due to anaerobic bacterial involvement.[193] In contrast, a study by Al Shahrani et al. indicated a lower incidence of thermal sensitivity, with only 75% of patients reporting similar complaints.[194] The chief complaints reported by participants provide valuable insights into the nature of orodental infections, particularly those involving obligate anaerobes. Tooth sensitivity to hot and cold, present in 100% of cases with obligate anaerobes, is likely due to nerve involvement or exposed dentin, suggesting deeper infections where anaerobes thrive in low-oxygen environments like necrotic tissues.[192] Toothache, reported in 93.3% of these cases, reflects inflammation and pressure buildup, which may be more severe due to the aggressive nature of anaerobic infections that often lead to tissue destruction and abscess formation.[193]The most significant association was with recurrent episodes of infection, seen in 46.6% of participants with obligate anaerobes compared to 22.9% without, highlighting the persistent nature of these infections. This recurrence may be due to ability of anaerobic bacteria to form biofilms or survive in necrotic tissue, making them harder to eliminate.[193,194] Other complaints, like pain while chewing or difficulty in swallowing were observed in both the groups of orodental infections with and without obligate anaerobes.

Diabetes mellitus was the most common risk factor observed among study participants. Significant difference was observed among cases with obligate anaerobic infection than those without obligate anaerobic infection.(p=0.04)[Table 12; Figure 5]

This is in concordance with the previously published studies. Jones et al. found that DM was prevalent in 30% of patients with anaerobic infections, supporting the current findings regarding the increased risk associated with diabetes.[195] According to Williams et al. a significant relationship between diabetes and anaerobic orodental infections was confirmed, where 25% of their patients with obligate anaerobes had DM.[196] Hernandez et al. reported that patients with periodontitis and a history of DM showed higher rates of obligate anaerobes, with a prevalence rate of 35%.[197] In a longitudinal study, White et al. found that a combination of systemic diseases, including DM, was associated with higher rates of orodental infections caused by anaerobic organisms.[198] Diabetes impairs the immune response by compromising the function of white blood cells, which are essential for combating infections.[195] Elevated blood sugar levels also hinder wound healing, as they lead to poor circulation and reduced oxygen delivery to tissues, creating an environment conducive to anaerobic bacteria proliferation.[195] Additionally, diabetes can alter the oral microbiome, favouring pathogenic bacteria, including obligate anaerobes, especially in individuals who experience dry mouth (xerostomia), a common condition in diabetics that decreases saliva's protective effects.[196] Furthermore, elevated glucose levels can lead to glycosylation of proteins, impairing immune function and tissue repair mechanisms.[196] The strong association between diabetes and periodontal disease increases the likelihood of anaerobic infections, as the inflammation and tissue destruction associated with periodontal disease create pockets that support anaerobic bacterial growth.[196] The prevalence of DM in patients with anaerobic infections highlights the need for careful monitoring of systemic conditions that may predispose individuals to oral infections.

In our study gram negative obligate anaerobes were the most common isolates obtained from both dental caries and periodontitis. *Prevotella spp* was identified as the most common gram negative anaerobes among prevotella spp.[Table13;Figure6] Prevotella oris was the commonest isolates obtained followed by prevotella buccae and prevotella oralis . Among gram positive obligate anaerobes, gram positive cocci were the predominating isolates obtained. Out of the 200 participants, 30 (15%) were identified with obligate anaerobic infections, with a total of 20 gram-negative and 10 gram-positive anaerobes detected. A study conducted by Smith et al. reported that gram negative obligate anaerobes were predominant in periodontitis patients, with a prevalence rate of 60%.[199] Study by Lee et al. cohort of 200

subjects, found that *Bacteroides fragilis* and *Prevotella oris* were significantly associated with periodontal disease, with 40% of periodontitis patients testing positive for these anaerobes.[200] A prospective study conducted by Jindal S et al.[201] investigated the anaerobic flora responsible for the orodental infections. The study revealed a significant predominance of anaerobic bacteria over aerobic bacteria, with anaerobes outnumbering aerobes by a 3-4 fold. Notably, anaerobic isolation rates were substantially higher in patients with Chronic Periodontitis (78.33%) compared to healthy subjects (48.33%), indicating a strong association between anaerobic microorganisms and the disease. Furthermore, obligate gram-negative anaerobes (70.21%) were predominantly isolated in patients with chronic periodontitis, outnumbering gram-positive anaerobes (14.89%). The most common anaerobic isolates in chronic periodontitis patients were *Prevotella spp. (60%), Fusobacterium sp. (50%), Porphyromonas sp. (30%), and Bacteroides sp. (25%)*.[202] These bacteria are naturally present in the oral cavity, where they coexist with facultative anaerobes and other microbial species.[201] Study by Thompson et al. reported the isolation of *Peptostreptococcus anaerobius* in 25% of patients with dental caries, aligning with findings from the current study.[202] Species such as *Prevotella* and *Fusobacterium* are particularly colonize the periodontal tissues and are known to produce virulence factors that facilitate tissue destruction and inflammation.[200] For instance, *Prevotella oris* and *Fusobacterium nucleatum* secrete enzymes that degrade host tissues, contributing to the breakdown of the periodontal ligament and the resorption of alveolar bone. [202] These anaerobes also engage in metabolic activities that produce various byproducts, such as short-chain fatty acids and toxins, which can further exacerbate inflammation and immune responses. [202]The interplay between these microbial products and the host's immune system can result in chronic inflammatory conditions, such as periodontitis, which may eventually lead to tooth loss if left untreated. [202] Additionally, the biofilm formation associated with anaerobes creates a protective environment that enhances their survival against antimicrobial agents, complicating treatment efforts.[201] Overall, the pathogenic potential of these obligate anaerobes is underscored by their ability to cause localized infections, promote chronic inflammation, and evade host defenses, making them significant contributors to orodental diseases.

In this study antimicrobial susceptibility testing for all the isolates was performed by E-strip method for determining the minimum inhibitory concentration (MIC).[Table 14] For representative anaerobic isolates[Table15] the results of E-strip method were also compared by agar dilution method as gold standard. [Table 16;Figure7] The results were categorised as susceptible and resistant. No intermediate sensitivity was reported for any of the isolates. The maximum sensitivity for both gram negative and gram positive isolates was observed for moxifloxacin followed by clindamycin and metronidazole. Poor susceptibility for other commonly prescribed antibiotics like metronidazole, tetracycline, and ampicillin hints towards which emerging antimicrobial resistance is a matter of concern and should be looked for. Study by Tashiro et al. noted 77% susceptibility to moxifloxacin and 65% to tetracycline in 35 anaerobic bacteria sourced from periodontal pockets, reinforcing the trend of high moxifloxacin susceptibility evident with the current study's findings.[203] Mendez et al. assessed 60 anaerobic isolates and found moxifloxacin to have 92% effectiveness, while ampicillin showed only 50% efficacy, echoing with the present study's results regarding the superior effectiveness of moxifloxacin.[204] Hsueh et al. confirmed high susceptibility rates of 88% and 82% for moxifloxacin and clindamycin, respectively, in their study of 50 anaerobes from dental infections, consistent with the present study's findings.[205] Overall, our study's findings reflect a consensus among various studies, demonstrating high susceptibility of obligate anaerobes to moxifloxacin and clindamycin, while highlighting the potential for regional variations in antibiotic resistance, particularly concerning ampicillin. The collective evidence underscores the reliability of clindamycin and moxifloxacin as effective treatment options for orodental infections caused by obligate anaerobes.

In the present study 100% concordance was observed between AST results by E-strip method with those of agar dilution method for clindamycin, metronidazole ,tetracycline and ampicillin except for moxifloxacin.[Table 17-21] isolate of *prevotella oralis* was found to be sensitive for moxifloxacin by E-strip & resistant by agar dilution method even after repeated testing. The possible explanation for the discordance results couldn't be conclusively explained. The AST results of moxifloxacin sensitivity for obligate anaerobes by E-strip method should be interpreted with cautions. Performance of AST by E-strip method is validated by determining it's sensitivity, specificity, PPV, NPV, & accuracy in comparison of E-strip method with agar dilution method as gold standard. The AST results were evaluated for all the five antimicrobial agents viz: moxifloxacin, clindamycin, metronidazole, tetracycline, ampicillin. 100% sensitivity, specificity, PPV, NPV was observed for clindamycin , metronidazole, tetracycline

and ampicillin except for moxifloxacin. Study by Hecth DW et al.[206] found that E-strip method shows high concordance with agar dilution method for certain anaerobic species such as *Bacteroides fragilis* and *Prevotella spp.* The overall percentage of concordance between the two methods was reported to be approximately 90%.[206]. Study by Schuetz et al compared the performance of the E-test and agar dilution methods in testing *Bacteroides fragilis* and *Prevotella* species against various antibiotics, including metronidazole and clindamycin. The study found a high level of concordance (approximately 92%) between the two methods for these antibiotics.[207] In a Study by Snydman et al *Bacteroides fragilis* group isolates were tested using both E-test and agar dilution for a range of antibiotics, including beta-lactams and metronidazole. The overall concordance between the two methods was reported at around 85-90%, with slight variability depending on the antibiotic tested.[208]

Although agar dilution method is recommended by CLSI for AST testing of anaerobes, its utility in routine diagnostic setup is limited due to requirement of technical expertise and long turnaround time. E-strip method is much simpler to perform even with less number of isolates in a resource limited setting's. The overall agreement suggest that E-strip method is a reliable alternative method of AST testing for agar dilution method for obligate anaerobes. However AST results for moxifloxacin may be confirmed by agar dilution method.

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SUMMARY

SUMMARY

- Among the 200 participants, obligate anaerobes were isolated in 30 participants (15%) and facultative anaerobes were isolated in 126 individuals (63%). The isolation rate of obligate anaerobes and facultative anaerobes in dental caries group is 14% and 64%. The isolation rate of obligate anaerobes and facultative anaerobes in periodontitis group is 16% and 62%.
- Majority of infections (58%) observed in the 19-36 age group, with the highest isolation rate for obligate anaerobes (7.5%). This was followed by the 37-54 age group (22%) and the 55-72 age group (11.5%)
- Significant difference in the orodental infections was observed among male to female ($p=<0.01$)
- Clinical symptoms of orodental infections were compared between patients with and without obligate anaerobes. Significant differences were observed for toothache ($p=0.05$) and recurrent episodes of infection ($p=0.01$)
- Diabetes mellitus was the most common risk factor observed in the study. A significant difference ($p=0.04$) was found between cases with obligate anaerobic infections and those without obligate anaerobic infection.
- Gram-negative obligate anaerobes were the most prevalent isolates, with *Prevotella species* being the most common followed by anaerobic gram positive cocci among both dental caries and periodontitis cases.
- AST of all isolates was performed using the E-strip method to determine the minimum inhibitory concentration (MIC).
- The AST results of E-strip were compared with the agar dilution method.
- 1 Isolate of *Prevotella spp* was susceptible by E-strip method and resistant by agar dilution method.
- 100% concordance was found between AST results obtained by the E-strip method and those from the agar dilution method for clindamycin, metronidazole, tetracycline, and ampicillin.
- Sensitivity , specificity, PPV ,NPV was 100% for clindamycin, Metronidazole, Tetracycline and ampicillin except moxifloxacin where sensitivity and NPV was 100%, specificity was 50%.

LIMITATIONS OF THE STUDY

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The present study suffers inherent limitation of small sample size. The relatively small sample size for obligate anaerobes may not adequately represent the diversity of orodental infections encountered in larger populations, as these infections can vary considerably across different regions and demographic groups. Additionally, the single centre nature of study restricts the variability of patient demographics ,microbial strains and antimicrobial resistance which can be captured by planning multicentric studies. Discordant results of E-strip with agar dilution for moxifloxacin resistance was not confirmed by genetic sequencing.

CONCLUSIONS

CONCLUSION

The study highlights the importance of obligate anaerobes in orodental infections. Infection is mostly polymicrobial along with facultative anaerobes particularly among young adults and males. Gram negative *Prevotella spp* are the predominant pathogens found to be associated with orodental infections followed by gram positive anaerobic cocci. E-strip method can be reliably used as suitable alternative of cumbersome and time consuming agar dilution method for detection of anaerobic antimicrobial resistance(AMR). Considering good sensitivity against moxifloxacin and clindamycin, these antimicrobials can be reliably used for empirical treatment and therapeutic management of these infections. Emerging antimicrobial resistance among obligate anaerobes is a matter of concern. Therefore, there is a need of large multicentric studies encompassing different geographical locations to generate cumulative antibiogram of obligate anaerobes for better understanding of antimicrobial resistance trends.

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ANNEXURES

CASE RECORD FORM

Date: Case no:
UHID No:

PERSONAL DETAILS

Age: Gender:
Address:
Occupation:
Date of hospital visit :
Clinical diagnosis :

S.no	Chief complaints	YES	NO
1	Toothache		
2	Pain/ discomfort with hot & cold temperature		
3	Pain/discomfort with pressure of chewing/biting		
4	Fever		
5	Swelling in face/cheek/neck		
6	Difficulty in swallowing		
7	Difficulty in breathing		
8	Any Other (Specify)		

S.no	Co-morbidities	YES	NO
1	Diabetes Mellitus		
2	Malignancies		
3	On steroid treatment		
4	Others (Specify)		

S.no	Past History	YES	NO
1.	H/o similar complaints in the past		
2.	H/o any invasive procedure in oral cavity in recent past		
	If Yes, Date of procedure: Duration of stay:		
3.	H/o taking any systemic antibiotic in the recent past		
	If yes; details :		
4.	Any others (Specify) :		

Socioeconomic status (as per modified Kuppuswamy Scale):
Sample type:

	Date and Time
Date of sample collection	
Time of sample collection	

SAMPLE PROCESSING (ANAEROBIC)

RCM turbidity after 48-72 hrs.: Yes ☐ No ☐

Colony characteristics

Brucella blood agar	Neomycin blood agar	Kanamycin Vancomycin laked blood agar

Identification :

Gram stain finding	
Aerotolerance test	
Facultative anaerobe identification	
Obligate anaerobe identification	
Conventional Method	
Automated Method	

Antimicrobial susceptibility pattern (obligate anaerobe)

E-strip Method

Sr.No	Antimicrobials	Susceptible	Intermediate	Resistant
1	Metronidazole			
2	Clindamycin			
3	Moxifloxacin			
4	Tetracycline			
5	Ampicillin			

Agar Dilution Method

Sr.No	Antimicrobials	Susceptible	Intermediate	Resistant
1	Metronidazole			
2	Clindamycin			
3	Moxifloxacin			
4	Tetracycline			
5	Ampicillin			

Participant Information Sheet
(For adult participants)

1. STUDY TITLE: Characterization and Antimicrobial resistance profile of obligate anaerobes associated with orodental infections.

2. STUDY INVESTIGATORS:

3. Student: Dr. Varshith.V, Junior Resident, Department of Microbiology

4. Guide: Dr. Shashwati Nema , Associate Professor, Department of Microbiology

5. Co-Guide/s:

a) Dr. Pankaj Goel, Professor and Head , Department of Dentistry

b) Dr. Arati Bhadade, Assistant Professor, Department of Microbiology

6. Introduction:

We are conducting a research study to know the antimicrobial resistance pattern of obligate anaerobic bacteria isolated from orodental infections for routinely prescribed antibiotics.. Beside the routine treatment and care at hospital, your sample will be collected as suggested by your clinician depending upon the type of infection for bacterial isolation and antimicrobial susceptibility testing. We would also like to collect data from your past medical records relevant for our study. If you agree to take part voluntarily, we will ask you to sign a consent form.

7. Purpose of the study:

The aim of our study is to analyse the distribution and antimicrobial resistance pattern of anaerobic bacteria obtained from patients with orodental infections. This will help us to find out the best antibiotics to be given to the patients suffering from orodental infections.

8.Taking part – what it involves:

a. Do I have to take part?

Participation in this research is entirely voluntary. A decision to not take part, or to withdraw at any time, will not affect your rights or medical care in any way.

b. What is expected of me if I take part?

If you participate, your dental sample will be collected depending upon the type of infection for anaerobic bacterial isolation and antimicrobial susceptibility testing. .

c. How long will my part in the study last?

Your participation in the study would last for 10 mins for sample collection during your visit to dental clinic. No multiple visits and follow-up required.

d. Do I have to come for extra visits or undergo extra investigations for the study?

No additional visits or extra sample will be required in the study.

e. What are the possible benefits in taking part?

The culture and sensitivity report for anaerobic bacterial isolates will be provided. This will guide your clinician for selection of right antimicrobial agent for your treatment.Taking part will help gain knowledge about the resistance of anaerobic bacteria causing orodental infections and best antibiotics for improved management and outcomes for patients with orodental infections . This will be beneficial for you as well as for the community at large.

f. What are the possible disadvantages and risks of taking part?

There are no major foreseeable disadvantages or risks because of the study. There may be risk of loss of privacy and confidentiality of information collected even though all possible measures will be taken to ensure safe data storage and access.

g. What happens to the information collected from me?

All information collected will be kept strictly confidential and not shared with anyone. Results from the study will be reported as group data and will not identify you in any way.

h. What if I change my mind regarding participation in the study?

You are entitled to change your mind and withdraw at any time during the study without any penalty or any change in your routine treatment and care.

i. Whom do I contact for more information or if I have further questions or concerns?

If you want any information or have questions or concerns, you can contact:

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प्रतिभागी सूचना पत्र

(वयस्क प्रतिभागियों के लिए)

1.अध्ययन का शीर्षक: ओरोडेंटल संक्रमण से जुड़े वाध्यकारी अवायवीय जीवों की विशेषता और रोगाणुरोधी प्रतिरोध प्रोफ़ाइल

2 अध्ययन जांचकर्ता

3 छात्र: डॉ. वर्षिथ. वी , जूनियर रेजिडेंट, माइक्रोबायोलॉजी विभाग

4 मार्गदर्शक :डॉ. शाश्वती नेमा , एसोसिएट प्रोफेसर, माइक्रोबायोलॉजी विभाग

5 सह मार्गदर्शक: डॉ. पंकज गोयल , प्रोफेसर और विभाग के प्रमुख , दंत चिकित्सा विभाग

सह मार्गदर्शक डॉ आरती भददे, असिस्टेंट प्रोफेसर , माइक्रोबायोलॉजी विभाग

6 परिचय:हम नियमित रूप से निर्धारित एंटीबायोटिक दवाओं के लिए ओरोडेंटल संक्रमण से पृथक अवायवीय जीवाणुओं के रोगाणुरोधी प्रतिरोध पैटर्न को जानने के लिए एक शोध अध्ययन कर रहे हैं। हम अपने अध्ययन के लिए प्रासंगिक आपके पिछले मेडिकल रिकॉर्ड से डेटा एकत्र करना चाहते हैं। अस्पताल में नियमित उपचार और देखभाल के अलावा, संक्रमण के प्रकार के आधार पर आपके चिकित्सक द्वारा सुझाए गए अनुसार आपका नमूना एकत्र किया जाएगा. यदि आप स्वेच्छा से भाग लेने के लिए सहमत हैं, तो हम आपसे एक सहमति फॉर्म पर हस्ताक्षर करने के लिए कहेंगे।

7 अध्ययन का उद्देश्य:हमारे अध्ययन का उद्देश्य ओरोडेंटल संक्रमण वाले रोगियों से प्राप्त एनारोबिक बैक्टीरिया के वितरण और रोगाणुरोधी प्रतिरोध पैटर्न का विश्लेषण करना है। इससे हमें ओरोडेंटल संक्रमण से पीड़ित मरीजों को दी जाने वाली सर्वोत्तम एंटीबायोटिक दवाओं का पता लगाने में मदद मिलेगी।

8 भाग लेना - इसमें क्या शामिल हैं?

a.क्या मुझे भाग लेना चाहिए?

इस अनुसंधान में भाग लेना पूर्णतः स्वैच्छिक है । भाग नहीं लेने के निर्णय से या किसी भी समय अपना नाम वापस लेने सेकिसी भी , रूप में आपके अधिकारों या आपकी चिकित्सा देखभाल पर कोई विपरीत प्रभाव नहीं पड़ेगा ।

b.यदि मैं इसमें शामिल होता हूं तो मुझसे क्या अपेक्षित हैं?

यदि आप भाग लेते हैं, तो हम अपने अध्ययन के लिए आपके पिछले मेडिकल रिकॉर्ड से (डेटा) एकत्र करेंगे। अस्पताल में नियमित उपचार और देखभाल के अलावा, संक्रमण के प्रकार के आधार पर आपका नमूना एकत्र किया जाएगा।

. cअध्ययन में मेरी भागीदारी कब तक चलेगी ?

दंत चिकित्सा क्लिनिक में आपकी यात्रा के दौरान नमूना संग्रह के लिए अध्ययन में आपकी भागीदारी 10 मिनट तक चलेगी। कोई एकाधिक विज़िट और फ़ॉलो-अप की आवश्यकता नहीं है।

. dक्या मुझे अध्ययन के लिए अतिरिक्त मुलाकातों के लिए आना होगा या अतिरिक्त जांच से गुजरना होगा?

अध्ययन में किसी अतिरिक्त मुलाकात या अतिरिक्त नमूने की आवश्यकता नहीं होगी।

.eइसमें शामिल होने से संभावित लाभ क्या है?

भाग लेने से ओरोडेंटल संक्रमण पैदा करने वाले अवायवीय बैक्टीरिया के प्रतिरोध के बारे में ज्ञान प्राप्त करने में मदद मिलेगी और बेहतर प्रबंधन के लिए सर्वश्रेष्ठ एंटीबायोटिक्स और ओरोडेंटल संक्रमण वाले रोगियों के लिए परिणाम मिलेंगे। यह आपके साथ-साथ पूरे समुदाय के लिए फायदेमंद होगा

£ . इसमें भाग लेने से संभावित हानि एवं जोखिम ।

अध्ययन के कारण कोई बड़ी या जोखिम नहीं हैं। एकत्र की गई जानकारी की गोपनीयता और गोपनीयता के नुकसान का जोखिम हो सकता है, भले ही सुरक्षित डेटा भंडारण और पहुंच सुनिश्चित करने के लिए सभी संभव उपाय किए जाएंगे।

.gमेरे बारे में एकत्र की गई सूचना का क्या किया जाएगा?

एकत्र की गई सभी सूचना पूर्णतः गोपनीय रखी जाएगी तथा अध्ययन से प्राप्त निष्कर्षों को अन्य किसी से साझा नहीं किया जाएगा।इन्हें ग्रुप आंकड़े के रूप मे रिपोर्ट किया जाएगा और किसी भी तरह से आपकी पहचान उजागर नहीं होगी ।

h.इस अध्ययन मे भाग लेने के लिए मेरे विचार बदलने पर क्या होगा?

अध्ययन के दौरान किसी भी समय बिना किसी क्षति या दंड के आपको अपने विचार बदलने का पूरा अधिकार है ।

i.इस संबंध मे अधिक जानकारी के लिए या बाद मे मेरे मन मे कोई प्रश्न या शंका होने पर मुझ किनसे संपर्क करना होगा?

यदि आप कोई सूचना चाहते हैं या आपका कोई प्रश्न है या शंका है तो आप इन नंबरों पर संपर्क कर सकते हैं:

डॉ.वर्षिथ.वी
स्नातक छात्र,
माइक्रोबायोलॉजी विभागभोपाल , एम्स ,
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डॉ. शशांक पुरवार
सदस्य सचिव,
संस्थान आचार समिति - छात्र अनुसंधान
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Participant Information Sheet

(For Pediatric participants)

1. STUDY TITLE: Characterization and Antimicrobial resistance profile of obligate anaerobes associated with orodental infections.

2. STUDY INVESTIGATORS:

3. Student: Dr. Varshith.V, Junior Resident, Department of Microbiology

4. Guide: Dr. Shashwati Nema , Associate Professor, Department of Microbiology

5. Co-Guide/s:

a) Dr. Pankaj Goel, Professor and Head, Department of Dentistry

b) Dr. Arati Bhadade, Assistant Professor, Department of Microbiology

6. Introduction:

We are conducting a research study to know the antimicrobial resistance pattern of obligate anaerobic bacteria isolated from orodental infections for routinely prescribed antibiotics.. Beside the routine treatment and care at hospital, sample of your ward will be collected as suggested by your clinician depending upon the type of infection for bacterial isolation and antimicrobial susceptibility testing. We would also like to collect data from past medical records relevant for our study. If you agree, we will ask you to sign a consent form.

7. Purpose of the study:

The aim of our study is to analyse the distribution and antimicrobial resistance pattern of anaerobic bacteria obtained from patients with orodental infections. This will help us to find out the best antibiotics to be given to the patients suffering from orodental infections.

8.Taking part – what it involves:

a. Why to take part?

Participation in this research is entirely voluntary. A decision to not take part, or to withdraw at any time, will not affect rights or medical care of your ward in any way.

b. What is expected of my ward's participation?

If you participate, the dental sample of your ward will be collected depending upon the type of infection for anaerobic bacterial isolation and antimicrobial susceptibility testing. .

c. How long will participation in the study last?

Your ward participation in the study would last for 10 mins for sample collection during your visit to dental clinic. No multiple visits and follow-up required.

d. Do I have to come for extra visits or undergo extra investigations for the study?

No additional visits or extra sample will be required in the study.

e. What are the possible benefits in taking part?

The culture and sensitivity report for anaerobic bacterial isolates will be provided. This will guide your clinician for selection of right antimicrobial agent for the treatment of your ward.Taking part will help gain knowledge about the resistance of anaerobic bacteria causing orodental infections and best antibiotics for improved management and outcomes for patients with orodental infections . This will be beneficial for the patient as well as for the community at large.

f. What are the possible disadvantages and risks of taking part?

There are no major foreseeable disadvantages or risks because of the study. There may be risk of loss of privacy and confidentiality of information collected even though all possible measures will be taken to ensure safe data storage and access.

g. What happens to the information collected from me?

All information collected will be kept strictly confidential and not shared with anyone. Results from the study will be reported as group data and will not identify you in any way.

h. What if I change my mind regarding participation in the study?

You are entitled to change your mind and withdraw at any time during the study without any penalty or any change in routine treatment and care of your ward.

i. Whom do I contact for more information or if I have further questions or concerns?

If you want any information or have questions or concerns, you can contact:

Dr. Varshith.V
Post graduate student,
Department of Microbiology, AIIMS Bhopal
(8975874713, varshithchowdary9@gmail.com)

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(7773010099,ihecpgr@aiimsbhopal.edu.in)

प्रतिभागी सूचना पत्र

(बाल चिकित्सा प्रतिभागियों के लिए)

.1अध्ययन का शीर्षक: ओरोडेंटल संक्रमण से जुड़े वाध्यकारी अवायवीय जीवों की विशेषता और रोगाणुरोधी प्रतिरोध प्रोफ़ाइल

.2अध्ययन जांचकर्ता

.3छात्र :डॉ. वर्षिथ. वी , जूनियर रेजिडेंट, माइक्रोवायोलॉजी विभाग

.4मार्गदर्शक : डॉ.शाश्वती नेमा , एसोसिएट प्रोफेसर, माइक्रोवायोलॉजी विभाग

.5सह मार्गदर्शक : डॉ. पंकज गोयल , प्रोफेसर और विभाग के प्रमुख , दंत चिकित्सा विभाग

सह मार्गदर्शक : डॉ आरती भददे, असिस्टेंट प्रोफेसर , माइक्रोवायोलॉजी विभाग

6. परिचय:हम नियमित रूप से निर्धारित एंटीवायोटिक दवाओं के लिए ओरोडेंटल संक्रमण से पृथक अवायवीय जीवाणुओं के रोगाणुरोधी प्रतिरोध पैटर्न को जानने के लिए एक शोध अध्ययन कर रहे हैं। हम आपके बच्चे का अध्ययन के लिए प्रासंगिक पिछले मेडिकल रिकॉर्ड से डेटा एकत्र करना चाहते हैं। अस्पताल में नियमित उपचार और देखभाल के अलावा, संक्रमण के प्रकार के आधार पर चिकित्सक द्वारा सुझाए गए अनुसार आपके बच्चे का नमूना एकत्र किया जाएगा. . यदि आप स्वेच्छा से भाग लेने के लिए सहमत हैं, तो हम आपसे एक सहमति फॉर्म पर हस्ताक्षर करने के लिए कहेंगे।

7 अध्ययन का उद्देश्य:हमारे अध्ययन का उद्देश्य ओरोडेंटल संक्रमण वाले रोगियों से प्राप्त एनारोबिक बैक्टीरिया के वितरण और रोगाणुरोधी प्रतिरोध पैटर्न का विश्लेषण करना है। इससे हमें ओरोडेंटल संक्रमण से पीड़ित मरीजों को दी जाने वाली सर्वोत्तम एंटीवायोटिक दवाओं का पता लगाने में मदद मिलेगी।

8 भाग लेना - इसमें क्या शामिल हैं?

a. क्यों भाग लेना चाहिए?

इस अनुसंधान में भाग लेना पूर्णतः स्वैच्छिक है । भाग नहीं लेने के निर्णय से या किसी भी समय अपना नाम वापस लेने सेकिसी भी , रूप मेंआपके बच्चे का अधिकारों या चिकित्सा देखभाल पर कोई विपरीत प्रभाव नहीं पड़ेगा ।

b.यदि इसमें शामिल होता हूं तो मेरे बच्चे का क्या अपेक्षित हैं?

हम अपने अध्ययन के लिए आपके पिछले मेडिकल रिकॉर्ड से (डेटा) एकत्र करेंगे। अस्पताल में नियमित उपचार और देखभाल के अलावा, संक्रमण के प्रकार के आधार पर आपका बच्चे का नमूना एकत्र किया जाएगा।

.cअध्ययन में भागीदारी कब तक चलेगी ?

दंत चिकित्सा क्लिनिक में आपकी यात्रा के दौरान नमूना संग्रह के लिए अध्ययन में आपकी भागीदारी 10 मिनट तक चलेगी। कोई एकाधिक विज़िट और फ़ॉलो-अप की आवश्यकता नहीं है।

.dक्या मुझे अध्ययन के लिए अतिरिक्त मुलाकातों के लिए आना होगा या अतिरिक्त जांच से गुजरना होगा?

अध्ययन में किसी अतिरिक्त मुलाकात या अतिरिक्त नमूने की आवश्यकता नहीं होगी।

.eइसमें शामिल होने से संभावित लाभ क्या है ?

भाग लेने से ओरोडेंटल संक्रमण पैदा करने वाले अवायवीय बैक्टीरिया के प्रतिरोध के बारे में ज्ञान प्राप्त करने में मदद मिलेगी और बेहतर प्रबंधन के लिए सर्वश्रेष्ठ एंटीवायोटिक्स और ओरोडेंटल संक्रमण वाले रोगियों के लिए परिणाम मिलेंगे। यह आपके बच्चे का साथ-साथ पूरे समुदाय के लिए फायदेमंद होगा

f.इसमें भाग लेने से संभावित हानि एवं जोखिम ।

अध्ययन के कारण कोई बड़ी या जोखिम नहीं हैं। एकत्र की गई जानकारी की गोपनीयता और गोपनीयता के नुकसान का जोखिम हो सकता है, भले ही सुरक्षित डेटा भंडारण और पहुंच सुनिश्चित करने के लिए सभी संभव उपाय किए जाएंगे।

.gमेरे बच्चे के बारे में एकत्र की गई सूचना का क्या किया जाएगा ?

एकत्र की गई सभी सूचना पूर्णतः गोपनीय रखी जाएगी तथा अध्ययन से प्राप्त निष्कर्षों को अन्य किसी से साझा नहीं किया जाएगा।इन्हें ग्रुप आंकड़े के रूप मे रिपोर्ट किया जाएगा और किसी भी तरह से आपकी पहचान उजागर नहीं होगी ।

h.इस अध्ययन मे भाग लेने के लिए मेरे विचार बदलने पर क्या होगा?

अध्ययन के दौरान किसी भी समय बिना किसी क्षति या दंड के आपको अपने विचार बदलने का पूरा अधिकार है ।

i.इस संबंध मे अधिक जानकारी के लिए या बाद मे मेरे मन मे कोई प्रश्न या शंका होने पर मुझ किनसे संपर्क करना होगा?

यदि आप कोई सूचना चाहते हैं या आपका कोई प्रश्न है या शंका है तो आप इन नंबरों पर संपर्क कर सकते हैं:

डॉ.वर्षिथ.वी
स्नातक छात्र,
माइक्रोवायोलॉजी विभागभोपाल , एम्स ,
8975874713, varshithchowdary9@gmail.com

डॉशशांक पुरवार .
सदस्य सचिव,
संस्थान आचार समिति - छात्र अनुसंधान
,7773010099ihecpgr@aiimsbhopal.edu.in

प्रतिभागी सूचना सहमति प्रपत्र

|PARTICIPANT INFORMED CONSENT FORM (PICF)|

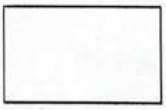
इस अध्ययन के लिए प्रतिभागी का पहचान नंबरः .....
Participant Identification Number for this Study: .....
अनुसंधान का शीर्षकः ओरोडेंटल संक्रमण से जुड़े बाध्यकारी अवायवीय जीवों की विशेषता और रोगाणुरोधी प्रतिरोध प्रोफ़ाइल
Title of Project : Characterization and Antimicrobial resistance profile of obligate anaerobes associated with orodental infections
शोधकर्ता छात्र का नाम : डॉ.वर्षिथ.वी फोन नं: ८९७५८७४७१३
Name of Student Investigator : Dr.Varshith.V Tel No: 8975874713
शोध मार्गदर्शक का नाम : डॉ शाश्वती नेमा
Name of Thesis Guide : Dr.Shashwati Nema

मैंने दिनांक 20.08.2022के सूचना पत्रक संस्करण II में दिए गए सभी तथ्यों को सावधानीपूर्वक पढ़ लिया है/ मुझे अपनी भाषा में स्पष्ट रूप से विस्तारपूर्वक समझा दिया गया है, और मैंने तथ्यों को पूर्णतः समझ लिया है। मैं पुष्टि करता/ करती हूँ, कि मुझे सवाल पूछने के अवसर प्रदान किए गए। The contents of the information sheet version II dated 20 August 2022 that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

मुझे इस अध्ययन की प्रकृति एवं उद्देश्य और इसके संभावित जोखिमों / लाभों तथा अध्ययन की अनुमानित अवधि एवं अन्य प्रासंगिक जानकारी के बारे में विस्तारपूर्वक समझा दिया गया है। मुझे पता है कि इस अध्ययन में मेरी भागीदारी स्वैच्छिक है तथा मैं इस अध्ययन से किसी भी समय बिना कोई कारण बताए, अपना नाम वापस लेने के लिए स्वतंत्र हूं और नाम वापस लेने पर मेरी चिकित्सा देखभाल या कानूनी अधिकारों पर कोई प्रभाव नहीं पड़ेगा। The nature and purpose of the study and its potential risks/benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

मैं समझता/समझती हूं कि इस अनुसंधान में मेरी भागीदारी से मेरे बारे में एकत्र की गई जानकारी और मेरे चिकित्सीय दस्तावेजों के किसी भी भाग को एम्स के जिम्मेदार व्यक्तियों द्वारा ही देखा जाएगा। मैं इन व्यक्तियों को मेरे रिकॉर्ड प्राप्त करने की अनुमति प्रदान करता/ करती हूँ। । understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from AIIMS. I give permission for this individuals to have access to my records.

यदि प्रतिभागी बच्चा है तो उसकी मौखिक या लिखित सहमति प्राप्त की गई है। If participants is child, his/her verbal of written assent has been obtained.
मैं उक्त अध्ययन में भाग लेने के लिए सहमत हूं/I agree to take part in the above study.

_____ 
(प्रतिभागी के हस्ताक्षर / बांए अंगूठे का निशान) (Signature/Left Thumb Impression)
दिनांक/Date.....स्थान/Place .....
प्रतिभागी का नाम/Name of the participant.....
पिता/पति/पत्नी का नाम /Son/Daughter/Spouse of.....
डाक का पूरा पता /Complete Postal Address .....
अगर अशिक्षित हों / If illiterate.
सूचना पत्र को प्रतिभागी को पढ़ा और समझाया गया है और प्रश्न पूछने का अवसर दिया गया है। मैं पुष्टि करता हूं कि व्यक्ति ने स्वतंत्र रूप से अपनी सहमति दी है।
The information sheet has been read and explained to the participant and given the opportunity to ask questions. I confirm that the individual has given consent freely.
कानूनी रूप से स्वीकार्य प्रतिनिधि या निष्पक्ष गवाह के हस्ताक्षर
Signature of Legally acceptable representative or impartial witness.
दिनांक/Date
एलएआर या निष्पक्ष गवाह का नाम / Name of LAR or impartial witness .
पता / Address .
प्रमाणित किया जाता है कि उक्त सहमति मेरी उपस्थिति में प्राप्त की गई।
This is to certify that the above consent has been obtained in my presence.

छात्र शोधकर्ता के हस्ताक्षर	दिनांक	स्थान
Signature of the student Investigator	Date	Place

Three copies should be made for (1) Patient , (2) Researcher, (3) Institution

THESIS NO	AGE	SEX	DIAGNOSIS	CHIEF COMPLAINTS							
				TOOTHACHE	PAIN WITH HOT & COLD TEMP	PAIN/DISCOMFORT WHILE CHEWING	FEVER	SWELLING OF FACE/NECK/CHEEK	DIFFICULTY IN SWALLOWING	DIFFICULTY IN BREATHING	ANY OTHER (SPECIFY)
TH001	56	F	PERIODONTITIS	NO	YES	NO	NO	NO	NO	NO	NO
TH002	30	M	PERIODONTITIS	NO	YES	NO	NO	NO	NO	NO	NO
TH003	47	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH004	20	F	PERIODONTITIS	NO	YES	NO	NO	NO	NO	NO	NO
TH005	47	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH006	46	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH007	27	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH008	21	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH009	24	M	DENTAL CARIES	NO	YES	NO	NO	NO	NO	NO	NO
TH010	31	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH011	68	M	PERIODONTITIS	NO	YES	NO	NO	NO	NO	NO	NO

TH012	29	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH013	40	F	PERIODONTITIS	NO	YES	NO	NO	NO	NO	NO	NO
TH014	37	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH015	21	M	DENTAL CARIES	NO	YES	NO	NO	NO	NO	NO	NO
TH016	50	F	PERIODONTITIS	NO	YES	NO	NO	NO	NO	NO	NO
TH017	57	M	PERIODONTITIS	NO	NO	NO	NO	NO	NO	NO	NO
TH018	29	F	PERIODONTITIS	NO	YES	NO	NO	NO	NO	NO	NO
TH019	31	M	PERIODONTITIS	NO	NO	YES	NO	NO	NO	NO	NO
TH020	22	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH021	40	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH022	69	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH023	54	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH024	39	M	DENTAL CARIES	YES	YES	YES	NO	NO	YES	NO	NO
TH025	61	F	DENTAL CARIES	YES	NO	NO	NO	NO	NO	NO	NO
TH026	40	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO

TH027	40	F	DENTAL CARIES	YES	NO	NO	NO	NO	NO	NO	NO
TH028	36	M	PERIODONTITIS	NO	NO	YES	NO	NO	NO	NO	NO
TH029	8	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH030	13	M	DENTAL CARIES	YES	YES	YES	NO	NO	YES	NO	NO
TH031	5	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH032	15	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH033	53	M	DENTAL CARIES	YES	NO	YES	YES	YES	NO	NO	YES
TH034	44	M	DENTAL CARIES	YES	NO	YES	NO	NO	NO	NO	NO
TH035	20	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH036	47	F	PERIODONTITIS	YES	NO	YES	NO	NO	NO	NO	NO
TH037	26	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH038	40	F	PERIODONTITIS	YES	NO	NO	NO	NO	NO	NO	NO
TH039	35	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH040	24	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH041	27	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO

TH042	21	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH043	66	F	PERIODONTITIS	NO	NO	YES	NO	NO	NO	NO	YES
TH044	36	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH045	23	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH046	26	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH047	26	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH048	23	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH049	22	F	PERIODONTITIS	NO	YES	NO	NO	NO	NO	NO	NO
TH050	25	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH051	23	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH052	63	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH053	19	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH054	36	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH055	24	M	DENTAL CARIES	YES	YES	YES	NO	NO	YES	NO	NO
TH056	27	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO

TH057	29	F	DENTAL CARIES	YES	YES	YES	NO	NO	YES	NO	NO
TH058	24	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	YES	NO
TH059	26	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH060	64	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH061	33	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	YES	NO
TH062	23	M	DENTAL CARIES	YES	YES	NO	NO	NO	YES	NO	NO
TH063	23	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH064	42	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH065	72	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH066	45	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH067	63	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH068	40	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH069	40	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH070	70	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH071	60	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO

TH072	32	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH073	45	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH074	62	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH075	30	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH076	34	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH077	40	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH078	40	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH079	47	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH080	83	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH081	46	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH082	46	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH083	37	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH084	25	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH085	61	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH086	24	M	DENTAL CARIES	YES	NO	YES	NO	NO	NO	NO	NO

TH087	24	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH088	25	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH089	23	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH090	26	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH091	28	M	DENTAL CARIES	NO	NO	YES	YES	NO	NO	NO	NO
TH092	22	F	PERIODONTITIS	YES	YES	YES	NO	NO	YES	NO	NO
TH093	23	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH094	20	M	DENTAL CARIES	YES	YES	YES	NO	NO	YES	NO	NO
TH095	58	M	PERIODONTITIS	YES	YES	YES	NO	NO	YES	NO	NO
TH096	20	F	DENTAL CARIES	YES	YES	YES	NO	NO	YES	NO	NO
TH097	61	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH098	65	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH099	29	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH100	29	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH101	37	M	PERIODONTITIS	NO	NO	NO	NO	NO	NO	NO	NO

TH102	30	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH103	30	F	PERIODONTITIS	NO	YES	NO	NO	NO	NO	NO	NO
TH104	56	F	PERIODONTITIS	NO	YES	NO	NO	NO	NO	NO	NO
TH105	27	M	DENTAL CARIES	NO	NO	YES	NO	NO	NO	NO	NO
TH106	18	F	DENTAL CARIES	YES	NO	NO	NO	YES	NO	NO	NO
TH107	36	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH108	24	F	PERIODONTITIS	NO	NO	NO	NO	NO	NO	NO	NO
TH109	24	F	DENTAL CARIES	NO	NO	NO	NO	NO	NO	NO	NO
TH110	28	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH111	23	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH112	25	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH113	22	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH114	22	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH115	36	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH116	36	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO

TH117	15	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH118	29	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH119	26	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH120	31	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH121	46	M	DENTAL CARIES	YES	YES	YES	YES	NO	NO	NO	NO
TH122	41	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH123	27	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH124	24	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH125	20	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH126	26	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH127	29	M	DENTAL CARIES	YES	NO	YES	NO	NO	NO	NO	NO
TH128	29	M	PERIODONTITIS	YES	NO	YES	NO	NO	NO	NO	NO
TH129	26	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH130	26	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH131	37	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO

TH132	37	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH133	21	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH134	17	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH135	47	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH136	54	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH137	54	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH138	50	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH139	30	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH140	30	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH141	45	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH142	30	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH143	30	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH144	45	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH145	45	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH146	31	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO

TH147	26	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH148	26	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH149	31	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH150	59	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH151	29	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH152	20	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH153	20	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH154	51	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH155	31	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH156	21	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH157	48	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH158	21	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH159	48	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH160	24	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH161	20	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO

TH162	64	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH163	30	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH164	14	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH165	14	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH166	22	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH167	22	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH168	18	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH169	18	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH170	25	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH171	25	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH172	53	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH173	53	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH174	14	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH175	14	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH176	30	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO

TH177	26	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH178	15	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH179	15	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH180	20	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH181	20	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH182	67	M	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH183	67	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH184	19	M	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH185	19	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH186	26	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH187	26	F	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH188	26	F	PERIODONTITIS	YES	YES	NO	NO	NO	NO	NO	NO
TH189	26	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH190	31	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH191	22	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO

TH192	13	M	DENTAL CARIES	YES	YES	YES	NO	NO	YES	NO	NO
TH193	24	F	PERIODONTITIS	YES	YES	YES	NO	NO	NO	NO	NO
TH194	25	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH195	64	M	DENTAL CARIES	YES	YES	YES	NO	NO	NO	NO	NO
TH196	26	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH197	29	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH198	28	F	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH199	38	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO
TH200	34	M	DENTAL CARIES	YES	YES	NO	NO	NO	NO	NO	NO

THESIS NO	CO-MORBIDITIES				PAST HISTORY				
	DIABETES MELLITUS	MALIGNANCIES	ON STEROID TREATMENT	OTHERS (SPECIFY)	H/O SIMILAR COMPLAINTS IN THE PAST	H/O INVASIVE PROCEDURE IN ORAL CAVITY	H/O TAKING SYSTEMIC ANTIBIOTICS	ANY OTHERS (SPECIFY)	SOCIOECONOMIC STATUS
TH001	NO	NO	NO	BLOOD PRESSURE	NO	NO	NO	NO	MIDDLE CLASS
TH002	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH003	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH004	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH005	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH006	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH007	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH008	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH009	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS

TH010	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH011	YES	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH012	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH013	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH014	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH015	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH016	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH017	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH018	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH019	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH020	NO	NO	NO	NO	NO	NO	NO	NO	UPPER MIDDLE CLASS
TH021	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS

TH022	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH023	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH024	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH025	YES	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH026	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH027	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH028	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH029	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH030	NO	NO	NO	NO	NO	NO	NO	NO	UPPER MIDDLE CLASS
TH031	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH032	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH033	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS

TH034	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH035	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH036	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH037	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH038	YES	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH039	NO	NO	NO	NO	NO	NO	NO	NO	UPPER MIDDLE CLASS
TH040	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH041	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH042	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH043	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH044	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH045	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS

TH046	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH047	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH048	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH049	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH050	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH051	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH052	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH053	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH054	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH055	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH056	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH057	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS

TH058	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH059	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH060	YES	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH061	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH062	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH063	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH064	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH065	YES	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH066	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH067	YES	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH068	YES	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH069	YES	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS

TH070	YES	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH071	YES	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH072	YES	YES (melanoma Treatment)	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH073	YES	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH074	YES	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH075	YES	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH076	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH077	YES	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH078	YES	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH079	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH080	YES	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH081	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS

TH082	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH083	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH084	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH085	YES	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH086	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH087	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH088	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH089	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH090	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH091	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH092	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH093	NO	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH094	NO	NO	NO	NO	YES	YES	YES	NO	LOWER MIDDLE CLASS

TH095	YES	NO	NO	NO	YES	YES	YES	NO	LOWER MIDDLE CLASS
TH096	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH097	YES	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH098	YES	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH099	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH100	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH101	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH102	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH103	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH104	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH105	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH106	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH107	YES	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS

TH108	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH109	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH110	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH111	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH112	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH113	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH114	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH115	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH116	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH117	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH118	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS

TH119	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH120	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH121	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH122	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH123	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH124	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH125	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH126	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH127	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH128	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH129	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH130	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS

TH131	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH132	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH133	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH134	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH135	YES	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH136	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH137	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH138	YES	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH139	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH140	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH141	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH142	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH143	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS

TH144	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH145	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH146	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH147	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH148	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH149	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH150	YES	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH151	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH152	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH153	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH154	YES	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH155	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH156	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH157	YES	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS

TH158	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH159	YES	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH160	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH161	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH162	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH163	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH164	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH165	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH166	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH167	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH168	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS

TH169	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH170	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH171	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH172	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH173	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH174	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH175	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH176	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH177	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH178	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS

TH179	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH180	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH181	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH182	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH183	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH184	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH185	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH186	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH187	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH188	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH189	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH190	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS

TH191	NO	NO	NO	NO	NO	NO	NO	NO	UPPER MIDDLE CLASS
TH192	NO	NO	NO	NO	NO	NO	NO	NO	UPPER MIDDLE CLASS
TH193	NO	NO	NO	NO	NO	NO	NO	NO	LOWER MIDDLE CLASS
TH194	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH195	YES	NO	NO	NO	YES	NO	NO	NO	LOWER MIDDLE CLASS
TH196	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH197	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH198	NO	NO	NO	NO	YES	NO	NO	NO	MIDDLE CLASS
TH199	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS
TH200	NO	NO	NO	NO	NO	NO	NO	NO	MIDDLE CLASS

MASTER CHART 3

THESIS NO	SAMPLE TYPE	D/O SAMPLE COLLECTION	RCM TURBIDITY	NO GROWTH	GRAM STAIN FINDING
TH001	PLAQUE	15/4/2023	YES	PIN HEAD MOIST GREY COLONIES	GPC in pairs
TH002	PLAQUE	19/4/2023	YES	WHITE PIN POINT COLONIES	GPC IN PAIRS
TH003	CARIOUS MATERIAL	19/4/2023	YES	PIN HEAD WHITE COLONIES	GPC IN PAIRS
TH004	PLAQUE	21/4/2023	YES	DRY PINPOINT WHITE COLONY	GPC IN PAIRS
TH005	CARIOUS MATERIAL	21/4/2023	YES	PIN HEAD WHITE COLONIES	GPC IN PAIRS
TH006	PLAQUE	25/4/2023	YES	GREY TRANSPARENT COLONIES	GNB
TH007	CARIOUS MATERIAL	26/4/2023	YES	PIN HEAD WHITE COLONIES	GPC IN PAIRS
TH008	CARIOUS MATERIAL	26/4/2023	YES	DRY FLAT IRREGULAR COLONY	BYC
TH009	CARIOUS MATERIAL	01-05-2023	YES	GREY FLAT COLONIES	GPC IN PAIRS
TH010	CARIOUS MATERIAL	01-05-2023	YES	WHITE SMALL COLONIES	GPC IN PAIRS
TH011	PLAQUE	01-05-2023	YES	GREENISH YELLOW COLONIES SEEN	GPC IN PAIRS
TH012	CARIOUS MATERIAL	01-05-2023	YES	WHITE ROUND PIN HEAD COLONY	GNB
TH013	PLAQUE	08-05-2023	YES	PIN POINT GREY COLONIES	GPC PAIRS
TH014	PLAQUE	08-05-2023	YES	WHITE FLAT COLONIES	GPC CHAINS AND PAIRS
TH015	CARIOUS MATERIAL	08-05-2023	YES	BIG WHITE MUCOID COLONIES	GNB (STRING TEST +)

TH016	PLAQUE	08-05-2023	YES	WHITE BIG COLONIES	GPC IN PAIRS
TH017	PLAQUE	08-05-2023	YES	WHITE BIG AND PIN POINT COLONIES	GPC IN PAIRS
TH018	PLAQUE	08-05-2023	YES	WHITE BIG COLONIES	GPC IN PAIRS
TH019	PLAQUE	09-05-2023	YES	NO GROWTH	N/A
TH020	PLAQUE	09-05-2023	YES	PIN HEAD WHITE COLONIES	GPC IN PAIRS
TH021	CARIOUS MATERIAL	09-05-2023	YES	PIN POINT COLONIES	GPB (STRING TEST -)
TH022	PLAQUE	09-05-2023	YES	DRY PINPOINT COLONIES	GPB (STRING TEST -)
TH023	PLAQUE	09-05-2023	YES	PIN POINT DRY COLONIES	GPB (STRING TEST -)
TH024	CARIOUS MATERIAL	09-05-2023	YES	PIN POINT COLONIES	GPC IN PAIRS
TH025	CARIOUS MATERIAL	07-06-2023	NO	PIN HEAD MOIST GREY COLONIES	GPC IN PAIRS AND CLUSTERS
TH026	CARIOUS MATERIAL	07-06-2023	YES	PIN POINT GREY COLONIES	GPC IN CUSTERS
TH027	CARIOUS MATERIAL	07-06-2023	YES	WHITE PIN POINT COLONIES	GPC IN CUSTERS
TH028	PLAQUE	07-06-2023	YES	YELLOWISH FLAT SPREADING COLONIES	GPC IN CUSTERS
TH029	PLAQUE	16-06-2023	YES	BETA HEMOYTIC WHITE COLONIES	GPC IN CUSTERS
TH030	CARIOUS MATERIAL	17-06-2023	YES	HEMOYTIC GREY PINHEAD COONIES	GPC IN CUSTERS
TH031	CARIOUS MATERIAL	17-06-2023	YES	BETA HEMOYTIC IRREGULAR SMALL COLONIES	GPC IN CUSTERS
TH032	CARIOUS MATERIAL	19-06-2023	YES	BETA HEMOYTIC ROUND COLONIES	GPC IN CUSTERS
TH033	CARIOUS MATERIAL	19-06-2023	NO	NO GROWTH	N/A
TH034	CARIOUS MATERIAL	19-06-2023	YES	WHITE ROUND BETA HEMOLYTIC COONIES	GPC IN CUSTERS

TH035	PLAQUE	21-06-2023	YES	PINPOINT WHITE COLONIES	GPC IN PAIRS AND CHAINS
TH036	PLAQUE	21-06-2023	YES	PINHEAD WHITE COLONIES	GPC IN CLUSTERS
TH037	PLAQUE	27-06-2023	NO	GREY PINHEAD COLONIES	GPC IN PAIRS AND CHAINS
TH038	PLAQUE	27-06-2023	YES	WHITE PINHEAD AND TRANSPARENT PINPOINT COLONIES	GPC IN PAIRS AND CHAINS
TH039	CARIOUS MATERIAL	27-06-2023	YES	PINHEAD WHITE COLONIES	GPC IN PAIRS
TH040	PLAQUE	27-06-2023	NO	HEMOLYTIC WHITE COLONIES	GPC IN PAIRS AND CHAINS
TH041	CARIOUS MATERIAL	28-06-2023	YES	PINPOINT GREY COLONIES	GPC IN PAIRS
TH042	PLAQUE	28-06-2023	YES	WHITE PINHEAD FLAT COLONIES	GPC IN PAIRS
TH043	PLAQUE	03-07-2023	YES	GREY AND YELOW PINPOINT COLONIES	GPC IN PAIRS
TH044	CARIOUS MATERIAL	03-07-2023	YES	WHITE PINPOINT FLAT COLONIES	GPC IN CHAINS AND PAIRS
TH045	PLAQUE	10-07-2023	YES	GREY TRANSPARENT PINPOINT COLONIES	GPC IN PAIRS AND SHORT CHAINS
TH046	PLAQUE	10-07-2023	NO	GREY PINPOINT IRREGULAR COLONIES	GPC IN CLUSTERS
TH047	PLAQUE	10-07-2023	YES	NO GROWTH	N/A
TH048	CARIOUS MATERIAL	11-07-2023	NO	YELLOWISH PINHEAD COLONIES	GPC IN CLUSTERS
TH049	PLAQUE	11-07-2023	NO	PINPOINT WHITE MOIST COLONIES	GPC IN CLUSTERS
TH050	CARIOUS MATERIAL	15-07-2023	NO	GREY SMALL PINPOINT COLONIES	GPC IN CHAINS AND CLUSTERS
TH051	CARIOUS MATERIAL	15-07-2023	YES	SMALL WHITE PINPOINT COLONIES	GPC IN CLUSTERS
TH052	PLAQUE	25-07-2023	YES	WHITE PINHEAD COLONIES	GPC PAIRS
TH053	PLAQUE	25-07-2023	NO	NO GROWTH	N/A
TH054	PLAQUE	25-07-2023	NO	SMALL WHITE PINPOINT COLONIES	GPC IN PAIRS AND SHORT CHAINS

TH055	CARIOUS MATERIAL	08-08-2023	YES	WHITE PINPOINT COLONIES	GPC IN PAIRS
TH056	CARIOUS MATERIAL	08-08-2023	YES	GREY PINPOINT MOIST COLONIES	GPC IN PAIRS/CHAINS
TH057	CARIOUS MATERIAL	08-08-2023	NO	SMALL GREY PINPOINT COLONIES	GPC IN PAIRS
TH058	CARIOUS MATERIAL	08-08-2023	YES	WHITISH GREY PINPOINT COLONIES	GPC IN PAIRS/CHAINS
TH059	CARIOUS MATERIAL	08-08-2023	YES	WHITE SMALL PINPOINT COLONY	GPC IN PAIRS
TH060	CARIOUS MATERIAL	21-08-2023	YES	PINPOINT DRY BROWN COLONIES	GPC IN CHAINS AND PAIRS
TH061	CARIOUS MATERIAL	21-08-2023	YES	TRANSPARENT WHITE DRY COLONIES	GPC IN PAIRS AND CLUSTERS
TH062	CARIOUS MATERIAL	21-08-2023	NO	WHITE PINPOINT COLONIES	GPC IN CHAINS
TH063	CARIOUS MATERIAL	21-08-2023	YES	NO GROWTH	N/A
TH064	CARIOUS MATERIAL	21-08-2023	YES	WHITE SMALL PINHEAD COLONIES	GPC CLUSTERS AND CHAINS
TH065	PLAQUE	17-09-2023	YES	SMALL TRANSPARENT PINPOINT COLONIES	GNB (STRING TEST +)
TH066	CARIOUS MATERIAL	17-09-2023	YES	MOIST SPREADING TRANSPARENT COLONIES	GNB (STRING TEST +)
TH067	PLAQUE	17-09-2023	YES	PIN HEAD WHITE COLONIES	GP C IN PAIRS AND SHORT CHAINS SEEN
TH068	PLAQUE	17-09-2023	YES	MOIST TRANSPARENT COLONIES	GNB (STRING TEST +)
TH069	CARIOUS MATERIAL	17-09-2023	YES	MOIST TRANSPARENT COLONIES	GNB (STRING TEST +)
TH070	PLAQUE	17-09-2023	YES	PIN POINT WHITE COLONIES	GPC CHAINS AND PAIRS
TH071	PLAQUE	17-09-2023	YES	WHITE MOIST SMALL COLONIES	GPC CHAINS AND PAIRS

TH072	PLAQUE	17-09-2023	YES	PINPOINT WHITE COLONIES	GPC CHAINS AND PAIRS
TH073	CARIOUS MATERIAL	17-09-2023	YES	PINPOINT WHITE COLONIES	GPC CLUSTERS
TH074	PLAQUE	17-09-2023	YES	SMALL TRANSPARENT COLONIES	GNB (STRING TEST +)
TH075	PLAQUE	17-09-2023	NO	NO GROWTH	N/A
TH076	PLAQUE	04-10-2023	YES	WHITE SMALL PINPOINT COLONIES	GPC CHAINS AND PAIRS
TH077	PLAQUE	04-10-2023	YES	WHITISH GREY MUCOID	GNB (STRING TEST +)
TH078	PLAQUE	04-10-2023	YES	MUCOID WHITE PINHEAD COLONIES	GNB (STRING TEST +)
TH079	PLAQUE	04-10-2023	YES	GREY MUCOID COLONIES	GNB (STRING TEST +)
TH080	PLAQUE	04-10-2023	YES	WHITE PIN HEAD COLONIES	GNB (STRING TEST +)
TH081	PLAQUE	16-10-2023	YES	PIN HEAD MOIST GREY COLONIES	GNB (STRING TEST +)
TH082	CARIOUS MATERIAL	16-10-2023	YES	NO GROWTH	N/A
TH083	PLAQUE	16-10-2023	YES	HEMOLYTIC WHITE COLONIES	GNB (STRING TEST +)
TH084	PLAQUE	25-10-2023	YES	SMALL PINHEAD MOIST COLONIES	GPC IN CLUSTERS
TH085	PLAQUE	25-10-2023	NO	NO GROWTH	N/A
TH086	CARIOUS MATERIAL	25-10-2023	YES	SMALL PINHEAD MOIST COLONIES	GPC CHAINS AND PAIRS
TH087	PLAQUE	25-10-2023	YES	PINPOINT SMALL MOIST COLONIES	GPC PAIRS AND CLUSTERS
TH088	CARIOUS MATERIAL	25-10-2023	YES	YELLOW MOIST PINHEAD COLONIES	GPC CLUSTERS
TH089	PLAQUE	25-10-2023	YES	SMALL PINPOINT BETA HEMOLYTIC COLONIES	GPC CHAINS AND PAIRS
TH090	CARIOUS MATERIAL	13-11-2023	YES	BIG WHITE PINHEAD COLONIES	GPC IN PAIRS AND CHAINS
TH091	CARIOUS MATERIAL	13-11-2023	YES	PIN HEAD MOIST GREY COLONIES	GPC IN CUSTERS AND PAIRS
TH092	PLAQUE	13-11-2023	YES	WHITE SMALL MOIST COLONIES	GPC IN CHAINS
TH093	CARIOUS MATERIAL	01-02-2024	YES	GREYISH WHITE PINPOINT COLONIES	GNB

TH094	CARIOUS MATERIAL	01-02-2024	YES	WHITE MOIST PINPOINT COLONIES	GNB
TH095	PLAQUE	01-02-2024	YES	WHITE PINHEAD MOIST COLONIES	GPC CLUSTERS
TH096	CARIOUS MATERIAL	22-02-2024	YES	GREY MOIST PINPOINT COLONIES	GPC CHAINS AND PAIRS
TH097	PLAQUE	22-02-2024	YES	WHITE MOIST PINPOINT COLONIES	GPC IN SHORT CHAINS
TH098	PLAQUE	22-02-2024	YES	GREYISH WHITE COLONIES	GPC IN TETRADS AND PAIRS
TH099	PLAQUE	22-02-2024	YES	NO GROWTH	N/A
TH100	CARIOUS MATERIAL	22-02-2024	NO	NO GROWTH	N/A
TH101	PLAQUE	06-03-2024	YES	SMALL PINPOINT COLONIES	GPB (STRING TEST -)
TH102	CARIOUS MATERIAL	15-03-2024	YES	WHITE SMALL PINPOINT COLONIES	GPC PAIRS AND CLUSTERS
TH103	PLAQUE	15-03-2024	YES	YELLOW PINPOINT COLONIES	GPC CLUSTERS AND PAIRS
TH104	PLAQUE	15-03-2024	YES	YELLOW PINPOINT COLONIES	GPC CLUSTERS
TH105	CARIOUS MATERIAL	15-03-2024	YES	WHITE SMALL MOIST COLONIES	GPC PAIRS
TH106	CARIOUS MATERIAL	15-03-2024	YES	WHITE PINHEAD MOIST COLONIES	GPC CLUSTERS AND CHAINS
TH107	CARIOUS MATERIAL	03-04-2024	YES	WHITE MOIST COLONIES	GPC CHAINS AND PAIRS
TH108	PLAQUE	03-04-2024	YES	FLAT AND ROUND TRANSPARENT COLONIES	GPC CHAINS AND PAIRS
TH109	CARIOUS MATERIAL	03-04-2024	YES	FLAT AND ROUND TRANSPARENT COLONIES	GPC CHAINS AND PAIRS
TH110	PLAQUE	03-04-2024	YES	WHITE SMALL PINPOINT COLONIES	GPC PAIRS
TH111	PLAQUE	03-04-2024	YES	BIG WHITE MOIST COLONIES	GPC IN CHAINS AND PAIRS
TH112	PLAQUE	03-04-2024	YES	PINPOINT GREY COLONIES	GPC IN PAIRS
TH113	PLAQUE	03-04-2024	YES	SMALL PINPOINT GREY COLONIES	GPC IN PAIRS AND SHORT CHAINS

TH114	PLAQUE	03-04-2024	YES	PINPOINT FLAT GREY COLONIES	GPC IN PAIRS AND SHORT CHAINS
TH115	PLAQUE	03-04-2024	YES	PINPOINT SMALL WHITE COLONIES	GPC IN PAIRS AND SHORT CHAINS
TH116	CARIOUS MATERIAL	03-04-2024	YES	SMALL GREY PINPOINT COLONIES	GPC IN PAIRS AND CHAINS
TH117	PLAQUE	03-04-2024	YES	SMALL GREY PINPOINT COLONIES	GPC IN PAIRS AND CHAINS
TH118	CARIOUS MATERIAL	03-04-2024	NO	NO GROWTH	N/A
TH119	CARIOUS MATERIAL	05-04-2024	YES	PINPOINT WHITE SMALL COLONIES	GNB
TH120	PLAQUE	05-04-2024	YES	GREY SMALL COLONIES	GPC IN PAIRS AND CHAINS
TH121	CARIOUS MATERIAL	05-04-2024	YES	GREY SMALL MOIST COLONIES	GP C IN PAIRS AND SHORT CHAINS SEEN
TH122	CARIOUS MATERIAL	05-04-2024	YES	MOIST GREY WHITE COLONIES	GNB
TH123	PLAQUE	05-04-2024	YES	PINPOINT FLAT COLONIES	GPC IN PAIRS AND CLUSTERS
TH124	CARIOUS MATERIAL	05-04-2024	YES	PINPOINT SMALL COLONIES	GPC IN PAIRS AND CLUSTERS
TH125	CARIOUS MATERIAL	05-04-2024	YES	PINPOINT TRANSPARENT COLONIES	GPC IN PAIRS AND CLUSTERS SEEN
TH126	CARIOUS MATERIAL	05-04-2024	YES	MOIST GREY COLONIES	GNB
TH127	CARIOUS MATERIAL	05-04-2024	YES	SMALL PINPOINT TRANSPARENT COLONIES	GPC IN PAIRS AND CHAINS
TH128	PLAQUE	05-04-2024	NO	NO GROWTH	N/A
TH129	PLAQUE	05-04-2024	YES	SMALL TRANSPARENT PINPOINT COLONIES	GPC CHAINS AND PAIRS
TH130	CARIOUS MATERIAL	05-04-2024	NO	NO GROWTH	N/A

TH131	PLAQUE	05-04-2024	YES	SMALL TRANPARENT COLONIES	GPC IN PAIRS AND SHORT CHAINS
TH132	CARIOUS MATERIAL	05-04-2024	NO	NO GROWTH	N/A
TH133	CARIOUS MATERIAL	05-04-2024	YES	SMALL WHITE TRANSPARENT COLONIES	GPC IN CHAINS AND PAIRS
TH134	CARIOUS MATERIAL	05-04-2024	NO	NO GROWTH	N/A
TH135	PLAQUE	08-05-2024	NO	NO GROWTH	N/A
TH136	CARIOUS MATERIAL	08-05-2024	YES	MOIST GREY COLONIES	GNB
TH137	PLAQUE	08-05-2024	NO	NO GROWTH	N/A
TH138	PLAQUE	08-05-2024	NO	NO GROWTH	N/A
TH139	PLAQUE	08-05-2024	NO	NO GROWTH	N/A
TH140	CARIOUS MATERIAL	08-05-2024	YES	GREY MOIST COLONIES, TRANSPARENT WHITE COLONIES	GNB, GPB
TH141	PLAQUE	08-05-2024	YES	TRANSPARENT WHITE DRY COLONIES	GPC CHAINS AND PAIRS
TH142	PLAQUE	08-05-2024	YES	WHITE MOIST PINHEAD COLONIES	GPC CLUSTERS
TH143	CARIOUS MATERIAL	08-05-2024	NO	NO GROWTH	N/A
TH144	PLAQUE	31-05-2024	YES	MOIST GREY COLONIES	GPC CLUSTERS AND PAIRS
TH145	CARIOUS MATERIAL	31-05-2024	NO	NO GROWTH	N/A
TH146	CARIOUS MATERIAL	31-05-2024	NO	NO GROWTH	N/A
TH147	CARIOUS MATERIAL	31-05-2024	NO	NO GROWTH	N/A
TH148	PLAQUE	31-05-2024	YES	WHITE FLAT MOIST COLONIES	GNB
TH149	PLAQUE	31-05-2024	NO	NO GROWTH	N/A

TH150	PLAQUE	06-06-2024	YES	SMALL WHITE TRANSPARENT COLONIES	GPC CLUSTERS
TH151	CARIOUS MATERIAL	06-06-2024	YES	GREYISH WHITE MOIST COLONIES	GPC PAIRS AND CLUSTERS
TH152	CARIOUS MATERIAL	06-06-2024	YES	GREYISH WHITE COLONIES	GPC CHAINS AND PAIRS
TH153	PLAQUE	06-06-2024	NO	NO GROWTH	N/A
TH154	PLAQUE	06-06-2024	YES	GREY MOIST COLONIES	GPC PAIRS AND TETRADS
TH155	CARIOUS MATERIAL	06-06-2024	YES	WHITE SMALL TRANSPARENT PINPOINT COLONIES	GPC CHAINS AND PAIRS
TH156	CARIOUS MATERIAL	14-06-2024	NO	NO GROWTH	N/A
TH157	PLAQUE	14-06-2024	NO	NO GROWTH	N/A
TH158	PLAQUE	14-06-2024	YES	WHITE MOIST PINPOINT COLONIES	GP C IN PAIRS AND SHORT CHAINS SEEN
TH159	CARIOUS MATERIAL	14-06-2024	YES	WHITE MOIST COLONIES	GPC CHAINS AND PAIRS
TH160	CARIOUS MATERIAL	14-06-2024	NO	NO GROWTH	N/A
TH161	CARIOUS MATERIAL	14-06-2024	NO	NO GROWTH	N/A
TH162	PLAQUE	14-06-2024	NO	NO GROWTH	N/A
TH163	CARIOUS MATERIAL	14-06-2024	YES	SMALL WHITE TRANSPARENT COLONIES	GPC IN PAIRS
TH164	CARIOUS MATERIAL	19-06-2024	NO	NO GROWTH	N/A
TH165	PLAQUE	19-06-2024	YES	GREY PINPOINT MOIST COLONIES	GPC PAIRS AND CLUSTERS
TH166	CARIOUS MATERIAL	19-06-2024	NO	NO GROWTH	N/A
TH167	PLAQUE	19-06-2024	NO	NO GROWTH	N/A

TH168	PLAQUE	19-06-2024	YES	GREY MOIST COLONIES	GPC IN PAIRS AND CHAINS
TH169	CARIOUS MATERIAL	19-06-2024	NO	NO GROWTH	N/A
TH170	CARIOUS MATERIAL	19-06-2024	NO	NO GROWTH	N/A
TH171	PLAQUE	19-06-2024	NO	NO GROWTH	N/A
TH172	PLAQUE	28-06-2024	YES	WHITE SMALL PINPOINT COLONIES	GPC IN PAIRS AND CHAINS
TH173	CARIOUS MATERIAL	28-06-2024	YES	GREY MOIST COLONIES	GPC IN PAIRS AND CHAINS
TH174	CARIOUS MATERIAL	28-06-2024	YES	WHITE FLAT MOIST COLONIES	GPC IN CHAINS AND PAIRS
TH175	PLAQUE	28-06-2024	NO	NO GROWTH	N/A
TH176	PLAQUE	28-06-2024	NO	NO GROWTH	N/A
TH177	PLAQUE	28-06-2024	NO	NO GROWTH	N/A
TH178	CARIOUS MATERIAL	24-08-2024	YES	GREY MOIST COLONIES	GPC IN PAIRS AND CHAINS
TH179	PLAQUE	24-08-2024	YES	SMALL WHITE TRANSPARENT COLONIES	GPC IN PAIRS
TH180	PLAQUE	24-08-2024	NO	NO GROWTH	N/A
TH181	CARIOUS MATERIAL	24-08-2024	NO	WHITE MOIST COLONIES	GPC IN PAIRS
TH182	PLAQUE	24-08-2024	YES	SMALL WHITE TRANSPARENT COLONIES	GPC IN PAIRS AND CHAINS
TH183	CARIOUS MATERIAL	24-08-2024	NO	WHITE MOIST COLONIES	GPC IN PAIRS
TH184	PLAQUE	24-08-2024	YES	GREY MOIST COLONIES	GPC IN PAIRS AND CHAINS
TH185	CARIOUS MATERIAL	24-08-2024	YES	WHITE SMALL TRANSPARENT PINPOINT COLONIES	GPC IN PAIRS
TH186	PLAQUE	24-08-2024	NO	NO GROWTH	N/A
TH187	CARIOUS MATERIAL	24-08-2024	NO	WHITE SMALL TRANSPARENT PINPOINT COLONIES	GPC IN PAIRS AND CHAINS

TH188	PLAQUE	24-08-2024	YES	SMALL WHITE TRANSPARENT COLONIES	GPC IN PAIRS AND CHAINS
TH189	CARIOUS MATERIAL	24-08-2024	YES	WHITE MOIST COLONIES	GPC IN CLUSTERS
TH190	CARIOUS MATERIAL	05-09-2024	YES	GREY MOIST COLONIES	GP C IN PAIRS AND SHORT CHAINS SEEN
TH191	CARIOUS MATERIAL	05-09-2024	YES	GREY MOIST COLONIES	GPC IN PAIRS
TH192	CARIOUS MATERIAL	05-09-2024	NO	NO GROWTH	N/A
TH193	PLAQUE	05-09-2024	NO	WHITE MOIST COLONIES	GPC IN PAIRS
TH194	CARIOUS MATERIAL	05-09-2024	NO	SMALL WHITE TRANSPARENT COLONIES	GPC IN PAIRS AND CHAINS
TH195	CARIOUS MATERIAL	05-09-2024	YES	GREY MOIST COLONIES	GPC IN PAIRS
TH196	CARIOUS MATERIAL	05-09-2024	NO	NO GROWTH	N/A
TH197	CARIOUS MATERIAL	05-09-2024	YES	SMALL WHITE TRANSPARENT COLONIES	GPC IN PAIRS AND CHAINS
TH198	CARIOUS MATERIAL	05-09-2024	NO	NO GROWTH	N/A
TH199	CARIOUS MATERIAL	11-09-2024	NO	WHITE MOIST COLONIES	GPC IN PAIRS
TH200	CARIOUS MATERIAL	11-09-2024	YES	GREY MOIST COLONIES	GPC IN PAIRS

THESIS NO	CATALASE	SC / TC	BE	AT TEST	FACULTATIVE ANAEROBE	OBLIGATE ANAEROBE by VITEK
TH001	POSITIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH002	NEGATIVE	N/A	NEGATIVE	NEGATIVE	N/A	PEPTONIPHILUS ASSACHAROLYTICUS
TH003	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH004	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH005	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH006	N/A	N/A	N/A	NEGATIVE	N/A	PREVOTELLA ORIS
TH007	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH008	N/A	N/A	N/A	N/A	N/A	N/A
TH009	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH010	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH011	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH012	N/A	N/A	N/A	NEGATIVE	N/A	PREVOTELLA ORIS
TH013	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH014	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH015	N/A	N/A	N/A	NEGATIVE	N/A	PREVOTELLA DISIENS
TH016	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH017	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH018	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH019	N/A	N/A	N/A	N/A	N/A	N/A
TH020	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH021	N/A	N/A	N/A	POSITIVE	N/A	N/A

TH022	N/A	N/A	N/A	POSITIVE	N/A	N/A
TH023	N/A	N/A	N/A	POSITIVE	N/A	N/A
TH024	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH025	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH026	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH027	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH028	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH029	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH030	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH031	POSITIVE	POSITIVE	N/A	POSITIVE	STAPHYLOCOCCUS AUREUS	N/A
TH032	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH033	N/A	N/A	N/A	N/A	N/A	N/A
TH034	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH035	NEGATIVE	N/A	NEGATIVE	NEGATIVE	N/A	PARVIMONAS MICRA
TH036	NEGATIVE	N/A	N/A	POSITIVE	STREPTOCOCCUS SPP	N/A
TH037	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH038	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH039	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH040	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH041	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH042	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH043	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH044	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A

TH045	NEGATIVE	N/A	NEGATIVE	NEGATIVE	N/A	PEPTONIPHILUS ASSACHAROLYTICUS
TH046	NEGATIVE	N/A	NEGATIVE	NEGATIVE	N/A	ANAEROCOCCUS PREVOTII
TH047	N/A	N/A	N/A	N/A	N/A	N/A
TH048	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH049	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH050	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH051	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH052	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH053	N/A	N/A	N/A	N/A	N/A	N/A
TH054	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH055	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP (STREPTOCOCCUS PYOGENES)	N/A
TH056	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP (STREPTOCOCCUS PYOGENES)	N/A
TH057	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH058	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH059	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP AGALACTIAE)	N/A
TH060	NEGATIVE	N/A	NEGATIVE	NEGATIVE	N/A	PEPTONIPHILUS ASSACHAROLYTICUS
TH061	NEGATIVE	N/A	NEGATIVE	NEGATIVE	N/A	PEPTOSTREPTOCOCCUS ANAEROBIUS
TH062	NEGATIVE	N/A	NEGATIVE	NEGATIVE	N/A	PARVIMONAS MICRA
TH063	N/A	N/A	N/A	N/A	N/A	N/A
TH064	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH065	POSITIVE	N/A	N/A	NEGATIVE	N/A	PREVOTELLA ORIS

TH066	POSITIVE	N/A	N/A	NEGATIVE	N/A	PREVOTELLA ORIS
TH067	NEGATIVE	N/A	N/A	POSITIVE	N/A	N/A
TH068	POSITIVE	N/A	N/A	NEGATIVE	N/A	PREVOTELLA ORIS
TH069	POSITIVE	N/A	N/A	NEGATIVE	N/A	PREVOTELLA ORIS
TH070	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH071	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH072	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH073	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH074	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	B FRAGILIS
TH075	N/A	N/A	N/A	N/A	N/A	N/A
TH076	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH077	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	PREVOTELLA ORIS
TH078	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	PREVOTELLA ORIS
TH079	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	CLOSTRIDIUM CLOSTRIDIOFORME
TH080	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	FUSOBACTERIUM NUCLEATUM
TH081	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	PREVOTELLA BUCCAE
TH082	N/A	N/A	N/A	N/A	N/A	N/A
TH083	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	PREVOTELLA BUCCAE
TH084	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH085	N/A	N/A	N/A	N/A	N/A	N/A
TH086	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH087	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH088	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A

TH089	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH090	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH091	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH092	NEGATIVE	N/A	NEGATIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH093	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	PREVOTELLA DISIENS
TH094	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	PREVOTELLA DISIENS
TH095	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH096	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH097	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH098	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH099	N/A	N/A	N/A	N/A	N/A	N/A
TH100	N/A	N/A	N/A	N/A	N/A	N/A
TH101	POSITIVE	N/A	N/A	POSITIVE	DIPTHEROIDS	N/A
TH102	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH103	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH104	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH105	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH106	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH107	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH108	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH109	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH110	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH111	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A

TH112	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH113	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH114	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH115	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH116	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH117	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH118	N/A	N/A	N/A	N/A	N/A	N/A
TH119	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	PREVOTELLA ORIS
TH120	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH121	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH122	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	PREVOTELLA BUCCAE
TH123	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	PARVIMONAS MICRA
TH124	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH125	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH126	POSITIVE	N/A	NEGATIVE	NEGATIVE	N/A	PREVOTELLA BUCCAE
TH127	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH128	N/A	N/A	N/A	N/A	N/A	N/A
TH129	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH130	N/A	N/A	N/A	N/A	N/A	N/A
TH131	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH132	N/A	N/A	N/A	N/A	N/A	N/A
TH133	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH134	N/A	N/A	N/A	N/A	N/A	N/A
TH135	N/A	N/A	N/A	N/A	N/A	N/A
TH136	POSITIVE	N/A	N/A	NEGATIVE	N/A	BACTERIOIDES OVATUS
TH137	N/A	N/A	N/A	N/A	N/A	N/A
TH138	N/A	N/A	N/A	N/A	N/A	N/A

TH139	N/A	N/A	N/A	N/A	N/A	N/A
TH140	POSITIVE	N/A	N/A	NEGATIVE	N/A	CLOSTRIDIUM CLOSTRIDIOFORME
TH141	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH142	POSITIVE	NEGATIVE	N/A	N/A	CONS	N/A
TH143	N/A	N/A	N/A	N/A	N/A	N/A
TH144	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH145	N/A	N/A	N/A	N/A	N/A	N/A
TH146	N/A	N/A	N/A	N/A	N/A	N/A
TH147	N/A	N/A	N/A	N/A	N/A	N/A
TH148	POSITIVE	N/A	N/A	NEGATIVE	N/A	PREVOTELLA ORIS
TH149	N/A	N/A	N/A	N/A	N/A	N/A
TH150	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH151	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH152	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH153	N/A	N/A	N/A	N/A	N/A	N/A
TH154	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH155	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH156	N/A	N/A	N/A	N/A	N/A	N/A
TH157	N/A	N/A	N/A	N/A	N/A	N/A
TH158	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH159	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS ANGINOSUS	N/A
TH160	N/A	N/A	N/A	N/A	N/A	N/A
TH161	N/A	N/A	N/A	N/A	N/A	N/A
TH162	N/A	N/A	N/A	N/A	N/A	N/A
TH163	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS ANGINOSUS	N/A
TH164	N/A	N/A	N/A	N/A	N/A	N/A

TH165	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH166	N/A	N/A	N/A	N/A	N/A	N/A
TH167	N/A	N/A	N/A	N/A	N/A	N/A
TH168	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH169	N/A	N/A	N/A	N/A	N/A	N/A
TH170	N/A	N/A	N/A	N/A	N/A	N/A
TH171	N/A	N/A	N/A	N/A	N/A	N/A
TH172	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH173	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH174	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH175	N/A	N/A	N/A	N/A	N/A	N/A
TH176	N/A	N/A	N/A	N/A	N/A	N/A
TH177	N/A	N/A	N/A	N/A	N/A	N/A
TH178	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH179	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH180	N/A	N/A	N/A	N/A	N/A	N/A
TH181	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH182	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH183	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH184	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH185	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH186	N/A	N/A	N/A	N/A	N/A	N/A
TH187	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH188	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH189	POSITIVE	POSITIVE	N/A	POSITIVE	STAPHYLOCOCCUS AUREUS	N/A
TH190	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A

TH191	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH192	N/A	N/A	N/A	N/A	N/A	N/A
TH193	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH194	NEGATIVE	N/A	NEGATIVE	POSITIVE	STREPTOCOCCUS SPP	N/A
TH195	NEGATIVE	N/A	POSITIVE	POSITIVE	ENTEROCOCCUS SPP	N/A
TH196	N/A	N/A	N/A	N/A	N/A	N/A
TH197	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A
TH198	N/A	N/A	N/A	N/A	N/A	N/A
TH199	NEGATIVE	N/A	N/A	POSITIVE	ENTEROCOCCUS SPP	N/A
TH200	POSITIVE	NEGATIVE	N/A	POSITIVE	CONS	N/A

THESIS NO	OBLIGATE ANAEROBE by VITEK	ANTIMICROBIAL SUSCEPTIBILITY TESTING									
		E STRIP METHOD					AGAR DILUTION				
		Metro	Clinda	Moxi	Tetra	Ampi	Metro	Clinda	Moxi	Tetra	Ampi
TH001	N/A										
TH002	PEPTONIPHILUS ASSACHAROLYTICUS	R	S	S	R	S	R	S	S	R	S
TH003	N/A										
TH004	N/A										
TH005	N/A										
TH006	PREVOTELLA ORIS	S	S	S	S	R					
TH007	N/A										
TH008	N/A										
TH009	N/A										
TH010	N/A										
TH011	N/A										
TH012	PREVOTELLA ORIS	S	S	S	S	R	S	S	S	S	R
TH013	N/A										
TH014	N/A										
TH015	PREVOTELLA DISIENS	S	S	S	S	S					
TH016	N/A										
TH017	N/A										
TH018	N/A										
TH019	N/A										

TH020	N/A										
TH021	N/A										
TH022	N/A										
TH023	N/A										
TH024	N/A										
TH025	N/A										
TH026	N/A										
TH027	N/A										
TH028	N/A										
TH029	N/A										
TH030	N/A										
TH031	N/A										
TH032	N/A										
TH033	N/A										
TH034	N/A										
TH035	PARVIMONAS MICRA	R	S	S	R	S					
TH036	N/A										
TH037	N/A										
TH038	N/A										
TH039	N/A										
TH040	N/A										
TH041	N/A										
TH042	N/A										
TH043	N/A										
TH044	N/A										
TH045	PEPTONIPHILUS ASSACHAROLYTICUS	R	S	S	R	S	R	S	S	R	S
TH046	ANAEROCOCCUS PREVOTII	S	R	S	R	S					

TH047	N/A										
TH048	N/A										
TH049	N/A										
TH050	N/A										
TH051	N/A										
TH052	N/A										
TH053	N/A										
TH054	N/A										
TH055	N/A										
TH056	N/A										
TH057	N/A										
TH058	N/A										
TH059	N/A										
TH060	PEPTONIPHILUS ASSACHAROLYTICUS	R	R	R	R	R					
TH061	PEPTOSTREPTOCOCCUS ANAEROBIUS	R	S	S	S	S					
TH062	PARVIMONAS MICRA	R	S	S	S	S					
TH063	N/A										
TH064	N/A										
TH065	PREVOTELLA ORIS	R	R	S	R	S	R	R	R	R	S
TH066	PREVOTELLA ORIS	R	S	R	R	S	R	S	R	R	S
TH067	N/A										
TH068	PREVOTELLA ORIS	S	S	S	S	S	S	S	S	S	S
TH069	PREVOTELLA ORIS	S	S	S	S	S	S	S	S	S	S
TH070	N/A										
TH071	N/A										
TH072	N/A										
TH073	N/A										

TH074	B FRAGILIS	S	S	S	S	R	S	S	S	S	R
TH075	N/A										
TH076	N/A										
TH077	PREVOTELLA ORIS	R	S	S	S	R	R	S	S	S	R
TH078	PREVOTELLA ORIS	R	S	S	S	R	R	S	S	S	R
TH079	CLOSTRIDIUM CLOSTRIDIOFORME	S	R	S	S	R	S	R	S	S	R
TH080	FUSOBACTERIUM NUCLEATUM	S	S	S	S	S					
TH081	PREVOTELLA BUCCAE	S	R	S	S	R					
TH082	N/A										
TH083	PREVOTELLA BUCCAE	S	S	S	S	S					
TH084	N/A										
TH085	N/A										
TH086	N/A										
TH087	N/A										
TH088	N/A										
TH089	N/A										
TH090	N/A										
TH091	N/A										
TH092	N/A										
TH093	PREVOTELLA DISIENS	S	S	S	S	R	S	S	S	S	R
TH094	PREVOTELLA DISIENS	R	S	S	S	R	R	S	S	S	R
TH095	N/A										
TH096	N/A										
TH097	N/A										
TH098	N/A										
TH099	N/A										
TH100	N/A										

TH101	N/A										
TH102	N/A										
TH103	N/A										
TH104	N/A										
TH105	N/A										
TH106	N/A										
TH107	N/A										
TH108	N/A										
TH109	N/A										
TH110	N/A										
TH111	N/A										
TH112	N/A										
TH113	N/A										
TH114	N/A										
TH115	N/A										
TH116	N/A										
TH117	N/A										
TH118	N/A										
TH119	PREVOTELLA ORIS	S	R	S	S	R	S	R	S	S	R
TH120	N/A										
TH121	N/A										
TH122	PREVOTELLA BUCCAE	S	S	S	S	S					
TH123	PARVIMONAS MICRA	S	R	S	R	S	S	R	S	R	S
TH124	N/A										
TH125	N/A										
TH126	PREVOTELLA BUCCAE	S	S	S	R	R					
TH127	N/A										

TH128	N/A										
TH129	N/A										
TH130	N/A										
TH131	N/A										
TH132	N/A										
TH133	N/A										
TH134	N/A										
TH135	N/A										
TH136	BACTERIOIDES OVATUS	R	S	R	R	R					
TH137	N/A										
TH138	N/A										
TH139	N/A										
TH140	CLOSTRIDIUM CLOSTRIDIOFORME	S	S	S	R	R					
TH141	N/A										
TH142	N/A										
TH143	N/A										
TH144	N/A										
TH145	N/A										
TH146	N/A										
TH147	N/A										
TH148	PREVOTELLA ORIS	S	S	S	R	R					
TH149	N/A										
TH150	N/A										
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TH200	N/A										